THE EFFECT OF 10- VERSUS 30-MINUTES OF ACUTE AEROBIC EXERCISE ON INSULIN AND GLUCOSE IN OBESE ADULTS

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Introduction: Obesity is associated with insulin resistance often accompanied by hyperinsulinemia and hyperglycemia, placing obese individuals at risk for type 2 diabetes and other chronic diseases. Acute exercise is associated with a decrease in postprandial insulin and glucose, but it is unknown how exercise of varying duration affects these variables. **Purpose:** The purpose of this study was to compare changes in postprandial insulin and glucose following 10-minutes (10-EX) and 30-minutes (30-EX) of aerobic exercise to a resting (REST) condition. **Methods:** 9 healthy, sedentary obese men and women (BMI: 33.6±3.2 kg/m^2; Age: 45.3±5.7 years) each performed 10-EX, 30-EX and REST in a randomized fashion. Blood was collected in the fasting state, and at 30-minute intervals following breakfast for 120-minutes. All exercise sessions were performed at 70-75% maximal heart rate. **Results:** Data was collected at all time points for five subjects, which were included in area under the curve (AUC) analyses. There was no significant difference in plasma insulin AUC between REST (12,270±6,148), 10-EX (10,633±5,162), and 30-EX (11,479±4,810) (p=0.354). There was no significant difference in plasma glucose AUC between REST (23,184±6,023), 10-EX (21,735±2,680), and 30-EX (22,899±3,328) (p=0.554). However, the patterns of change were not consistent across experimental conditions. A significant main effect for condition (p=0.018) was detected at 60-MIN for insulin, with lower insulin observed in 30-EX (32.3±12.3) compared to 10-EX (66.8±30.3) or REST (94.9±48.0). Insulin rebounded following exercise and was significantly higher at 120-MIN in 30-EX (74.3±45.2) compared to REST (55.4±45.7) (p=0.042). Plasma

glucose followed a similar pattern, and a significant main effect was observed at 90-MIN ($p=0.037$) at was elevated in 30-EX (147.6±29.0) compared to 10-EX (122.2±21.5) or REST (121.4±38.0). **Conclusion:** There was no significant difference in plasma insulin or glucose AUC between 10-EX, 30-EX and REST, but pattern of change was not consistent across conditions. Additional research should explore if differences in pattern of change following 10- or 30-minutes of exercise affect health outcomes in obese adults.

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1.0 INTRODUCTION

1.1 OBESITY AND INSULIN RESISTANCE

The prevalence of obesity in the United States has reached epidemic proportions, with an estimated 33.8% of adults classified as obese as of 2008.[1] Obesity is associated with increased risk for several chronic diseases including type 2 diabetes mellitus, cardiovascular disease, certain forms of cancer, sleep apnea and osteoarthritis.[2] While the etiology of obesity is complex, the underlying cause is excess of energy consumed beyond what is expended. This excess energy is stored as fat in adiopocytes, resulting in adipocyte hypertrophy and hyperplasia and causing dysfunction of adiopocytes. This adipocyte dysfunction appears to be the mechanism responsible for initiating a multitude of obesity-associated health consequences, including insulin resistance.[3]

Insulin resistance is defined as a state in which a given amount of insulin produces a less than normal biological response, and is considered a major risk factor associated with obesity.[4] Insulin resistance and accompanying hyperinsulinemia increase risk for several chronic diseases, with a particularly strong link to diabetes and cardiovascular disease. As a patient becomes insulin resistant, β-cells in the pancreas compensate by producing extra insulin to maintain blood glucose. Over time, β-cells may begin to produce less insulin or ultimately cease insulin production. This β-cell failure results in hyperglycemia and eventually leads to type 2 diabetes.

Obesity appears to contribute to insulin resistance by two primary mechanisms: increased circulation of free fatty acids (FFA)[5] and inflammation associated with adipocyte dysfunction.[6-7] The excess adipose tissue that leads to obesity results in elevated rates of lipolysis, causing increased concentrations of FFAs in the bloodstream. Increased plasma FFA attenuates insulin-stimulated glucose uptake[8] and increases gluconeogenesis by the liver, resulting in a state of increased glucose production and decreased glucose uptake.[2]

Obesity is also associated with increased systemic inflammation, which also contributes to insulin resistance. Chronic excessive energy intake leads to adipoctye enlargement, causing the endoplasmic reticulum to trigger the unfolded protein response (UPR). This initiates an inflammatory response and macrophage activation.[6] The inflammatory mediators secreted as part of the inflammatory response are thought to contribute to insulin resistance.[9]

1.2 HEALTH CONSEQUENCES OF HYPERINSULINEMIA

Insulin resistance is a health concern as it may progress to β-cell insufficiency or failure, resulting in the development of type 2 diabetes. However, hyperinsulinemia that exists early in the development of insulin resistance is associated with health complications and development of chronic disease that is not limited to type 2 diabetes. There is evidence specifically linking insulin resistance and hyperinsulinemia with cardiovascular disease, hypertension, hyperlipidemia, non-alcohol fatty liver disease, and polycystic ovarian syndrome.

Fasting insulin has been reported to predict cardiovascular disease risk. In a cohort of men and women aged 25-64 from the San Antonio Heart Study, elevated fasting insulin was associated with increased risk for cardiovascular disease.[10] Additionally, in a group of non-

diabetic older men, risk of cardiovascular events was greater among individuals in the highest quartile of fasting plasma insulin when compared to those in lower quartiles.[11] Accelerated progression of atherosclerosis is associated with high fasting insulin, and hyperinsulinemia may also predict more complex atherosclerotic lesions.[12] The increased rate and complexity in atherosclerotic developement in patients with high fasting insulin provides a potential mechanistic link between hyperinsulinemia and increased risk for cardiovascular disease.

High fasting insulin is associated with several cardiovascular disease risk factors, particularly dyslipidemia and hypertension. Hyperinsulinemia may contribute to high triglyceride levels and low concentrations of HDL cholesterol.[13] Elevated insulin levels are also common in hypertensive patients. A prospective study of middle-aged men reported that fasting insulin and insulin response to an oral glucose tolerance test (OGTT) were significant risk factors for the development of hypertension.[14] Thus, it is possible that insulin plays a role in the pathogenesis of hypertension and dyslipidemia, which may also explain the association between increased risk of cardiovascular disease and insulin resistance.

Hyperinsulinemia also contributes to the development of other conditions, particularly non-alcoholic fatty liver disease (NAFLD) and polycystic ovarian syndrome (PCOS). For example, in a 5-year prospective study, patients with increased fasting insulin were at higher risk for NAFLD compared to patients with lower fasting insulin levels.[15] Additionally, insulin resistance and accompanying hyperinsulinemia is also considered an integral component in the pathogenesis of PCOS.[16]

While obesity plays a critical role in the development of insulin resistance, resulting hyperinsulinemia may accelerate weight gain or inhibit weight loss. The role of insulin is to facilitate glucose uptake for use and storage in the tissues, and insulin levels are reported to

predict weight gain over time.[17-18] Insulin resistance is associated with obesity, and hyperinsulinemia may perpetuate weight gain, creating a cycle of increasing levels of obesity and insulin resistance.

1.3 EFFECT OF EXERCISE ON INSULIN AND GLUCOSE

It is important to explore therapeutic methods for decreasing insulin resistance and hyperinsulinemia to delay the onset of diabetes and cardiovascular disease, and prevent the additional health consequences associated with insulin resistance. Exercise is one purported method for decreasing insulin resistance. Both acute and chronic exercise is associated with improvements in insulin sensitivity, and decreased circulating insulin and glucose.

A single bout of moderate-intensity aerobic exercise has also been shown to improve insulin sensitivity among adults with varying degrees of insulin resistance. Improved insulin resistance has been reported in healthy men and women when measured 12-hours following a one- to two-hour bout of aerobic activity performed at 60% VO_{2peak}.[19] Improved insulin action has also been observed in both diabetic and nondiabetic obese adults. Cusi et al. reported that in a group of obese nondiabetics, one hour of aerobic exercise at 65% VO_{2max} increased insulin stimulated glucose uptake during a hyperinsulinemic euglycemic clamp.[20] Similarly, Burstein et al. observed an improved insulin response during a hyperinsulinemic euglycemic clamp following 45 minutes of exercise at 50% VO_{2max} in both diabetic and nondiabetic obese adults.[21] Acute exercise is also associated with decreased postprandial plasma insulin and glucose in diabetics. For example, Larsen et al. had older men with diabetes exercise for 45 minutes at 50% VO_{2max} after consuming breakfast, and observed significant decreases in glucose and insulin

throughout the post-exercise period.[22] These studies collectively indicate that a single session of continuous, aerobic exercise acutely improves insulin resistance and decreases plasma insulin and glucose concentrations.

The effect of exercise on insulin and glucose is transient, lasting for a period of 2 to 72 hours following exercise.[23-25] King et al. observed this short-tem improvement in insulin sensitivity in a group of trained subjects. These subjects completed five consecutive days of aerobic training, followed by seven days of inactivity. Insulin sensitivity was improved one and three days following exercise, but returned to baseline values by the fifth day of inactivity.[23] Similar results were reported by the HERITAGE study. Following a 6-week aerobic training program, fasting insulin levels were significantly lower 24-hours following the final exercise session but had returned to baseline by 72 hours post-exercise.[25]

Current health recommendations suggest that adults perform a minimum of 30-minutes of moderate intensity aerobic exercise on most days of the week. These guidelines also state that aerobic activity does not need to be performed in one continuous session, but may be accumulated in 10-minute bouts. [26-28] Performing multiple sessions in minimum 10-minute bouts appears to have a similar effect on weight[29-31] and cardiovascular fitness[29-36] as performing one continuous exercise bout. However, the effectiveness of short bouts for improving other health outcomes such as insulin and glucose is currently unknown.

Although the effect of short bouts for decreasing insulin and glucose has not yet been determined, guidelines for exercise training in populations characterized by hyperinsulinemia and hyperglycemia (e.g., obese and diabetic individuals) suggest accumulating short bouts to meet physical activity requirements.[24, 28] However, the chronic effect of accumulating short bouts of exercise on insulin and glucose is not yet understood, and the limited research on this

topic has presented equivocal results. For example, Donnelly et al. reported a decrease in both fasting insulin and insulin response to an OGTT following 18 months of exercise training consisting of two, 15-minute sessions performed 5 days per week.[32] Conversely, Eriksen et al. recently reported that fasting plasma glucose and glucose response to an oral glucose tolerance test (OGTT) were significantly lower following intermittent exercise training compared to continuous training. However, there was no change in insulin in either group.[37] Based on these two studies, the efficacy of intermittent exercise for decreasing insulin and glucose in obese adults is unclear, indicating a need for additional research.

While it is possible that accumulating exercise in multiple bouts decreases insulin and glucose as effectively as continuous exercise training, few studies have explored whether there is a difference in these sessions following acute exercise. Most studies investigating the acute effect of intermittent versus continuous exercise have done so in a healthy, normal weight population and performed measurements of insulin resistance 12 or more hours following the final exercise bout.[38-39] Currently only one study has examined the acute effect of short bouts of exercise in a population that included obese subjects.[40] However, this study included subjects with a BMI range from healthy to obese (23.9kg/m^2 to 34.5 kg/m^2), making it difficult to generalize these results specifically to an obese population. Therefore, the insulin and glucose response immediately following short bouts of exercise compared to continuous exercise is currently unknown in an obese population. It is possible that multiple short bouts of activity result in transient improvements in hyperinsulinemia and hyperglycemia that are additive and more effective for decreasing insulin and glucose compared to one single bout of equal duration. However, no study has yet explored this question.

1.4 GAPS IN THE LITERATURE

Exercise training is an effective means for decreasing insulin and glucose. However, the literature does not currently indicate if accumulating short bouts of exercise is as effective as one continuous bout for decreasing plasma insulin and glucose. Few studies have examined this topic, and methodological differences make interpretation of results difficult. In order to most effectively explore the effect of accumulating short bouts of exercise, it would be helpful to know how a single 10-minute bout of aerobic activity affects measures of insulin and glucose. It is possible that a single ten-minute bout does not improve insulin or glucose beyond what is observed in a resting state, and that performing short bouts throughout the day would have little impact on insulin and glucose concentrations. However, 10-minutes of exercise may significantly improve insulin and glucose, suggesting that multiple bouts may be preferable to one 30-minute session. Therefore, the aim of this study was to determine the effect of a 10-minute and 30-minute exercise session compared to a resting condition on changes in insulin and glucose.

1.5 SPECIFIC AIMS

The specific aims of this study were:

1. To compare the effect of three conditions [30-minutes of aerobic exercise (30-EX), 10-minutes of aerobic exercise (10-EX) or a resting condition (REST)] on postprandial plasma insulin over a 2-hour observation period.

2. To compare the effect of three conditions [30-EX, 10-EX, REST] on postprandial plasma glucose over a 2-hour observation period.

1.6 HYPOTHESES

1. It was hypothesized that the 10-EX condition would result in a greater decrease in postprandial insulin compared to the REST condition.

2. It was hypothesized that the 30-EX condition would result in a greater decrease in postprandial insulin compared to the 10-EX condition.

3. It was hypothesized that the 10-EX condition would result in a greater decrease in postprandial glucose compared to the REST condition.

4. It was hypothesized that the 30-EX condition would result in a greater decrease in postprandial insulin compared to the 10-EX condition.

1.7 CLINICAL RATIONALE

The clinical rationale for this study was that the effect of short bouts of exercise, defined as ten minutes, is currently unknown. This study was the first two address the question of whether 10 minutes of exercise effectively decreases insulin and glucose compared to a resting condition. If there was no significant change in these measures compared to rest, it is unlikely that accumulating 10 minute bouts would result in significant changes in insulin and glucose. It is also possible that 10 minutes of exercise significantly decreases insulin and glucose compared to rest, but the magnitude is not as great as what is observed following 30-minutes of exercise. In this instance, it is possible that performing multiple 10-minute sessions would have an equal or greater effect as performing a single, longer exercise session for managing insulin and glucose. Finally, it is possible that 10 minutes of exercise results in a decrease in insulin and glucose that is similar in magnitude as a 30-minute session. If this effect was observed, it is likely that accumulating exercise in 10-minute bouts would result in significantly greater improvements in insulin and glucose than performing a single, longer session. Therefore, this study provides the initial data necessary to further explore the effectiveness of accumulating shout bouts of exercise to manage insulin and glucose levels in an obese population.

2.0 REVIEW OF THE LITERATURE

2.1 INTRODUCTION

Obesity is associated with hyperinsulinemia and insulin resistance, predisposing individuals to health complication such as cardiovascular disease, NAFLD, PCOS, and continued weight gain. Exercise is one treatment for improving insulin resistance. Both acute and chronic exercise is associated with decreased insulin and glucose and improvements insulin resistance.

Current physical activity recommendations suggest that aerobic activity can be accumulated in shout bouts lasting a minimum of 10-minutes. However, it is not clear if accumulating short bouts effectively decreases insulin and glucose. It is also unknown if short bouts of exercise produce changes in insulin that are equal to a longer bout of exercise. Current research examining changes the effect of short bouts of exercise on insulin and glucose is limited and results are conflicting.[32, 37] Therefore it is necessary to further examine the role of short bouts of exercise in normalizing insulin levels in obese adults.

2.2 OBESITY AND INSULIN RESISTANCE

The prevalence of obesity in the United States has reached epidemic proportions, with an estimated 33.8% of adults classified as obese as of 2008.[1] Obesity is a public health concern as it

associated with increased risk for many chronic diseases including type 2 diabetes mellitus, cardiovascular disease, certain forms of cancer, sleep apnea and osteoarthritis.[2] Risk of all cause mortality also increases with greater levels of overweight and obesity.[41] There is a particularly strong link between obesity and type 2 diabetes; current estimates are that 82% of diagnosed diabetics are overweight and 55% are classified as obese.[42]

The connection between obesity and diabetes appears to be obesity-mediated insulin resistance. Insulin resistance is defined as a state in which a given amount of insulin produces a reduced biological response.[4] Initially, the β-cells of the pancreas respond to insulin resistance by producing extra insulin to maintain normal blood glucose levels, resulting in chronic hyperinsulemia. Over time the high output of insulin may cause β-cell failure and deterioration in glucose homeostasis; at this point an individual would be diagnosed with type 2 diabetes. While prevention of diabetes is a concern, high insulin levels accompanying insulin resistance which precedes β-cell failure also negatively impact other health outcomes.[5, 13] Therefore, it is necessary to explore methods for decreasing elevations in insulin, which accompany insulin resistance in non-diabetic populations.

Obesity is associated with fasting hyperinsulinemia, decreased insulin-stimulated glucose uptake, and decreased suppression of hepatic glucose production.[43-45] The mechanisms contributing to obesity-induced insulin resistance are increased circulation of free fatty acids (FFA), ectopic fat distribution, and inflammation associated with adipocyte dysfunction. These initial pathologies cause further metabolic disturbances within insulin sensitive tissues, including skeletal muscle, adipocytes and hepatocytes. As these tissues becomes insulin resistant and are unable to properly utilize and store glucose for energy, β-cell insulin production continues to

increase to maintain glucose homeostasis. The result is chronic hyperinsulinemia that may progress to β-cell failure and hyperglycemia over time.

2.2.1 Increased FFA

Obesity is associated with increased plasma FFA,[43, 46-47] especially in the presence of elevated abdominal adiposity.[47] Adipocytes are resistant to the anti-lipolytic effect of insulin in obese individuals, resulting in elevated circulating FFA levels.[6, 47] Jensen et al. reported a reduced suppression in FFA turnover during a eugylcemic and hyperinsulinemic clamp in healthy, obese women compared to normal weight women.[47] Elevated FFA compromise fuel utilization by decreasing insulin-stimulated glucose uptake. Decreased insulin-stimulated carbohydrate uptake has been observed following lipid infusion in healthy adults[8, 48-50] and type 2 diabetics.[51] Belfort et al., reported that increasing plasma FFA in a group of lean, healthy adults to levels comparable to obese individuals significantly decreased insulin-stimulated glucose uptake. [50] Therefore, increased levels of FFA found in obesity are thought to contribute to insulin resistance.

2.2.2 Ectopic fat distribution

Chronic elevations in FFA and lipid oversupply due to obesity result in deposition of fatty acids in tissues besides adiopocytes, including skeletal muscle and the liver. This ectopic fat deposition is thought to disrupt the normal function of these tissues, resulting in insulin resistance.[52] Levels of skeletal muscle triglyceride are elevated in obese individuals,[45, 53] contributing to insulin resistance.[45, 54-55] Goodpaster et al. reported that increased amounts of adipose tissue deposited within skeletal muscle was significantly associated with insulin

resistance in obese, glucose tolerant men and women.[45] Similarly, Pan et al. demonstrated that in a group of 38 non-diabetic male Pima Indians, higher concentrations of muscle triglyceride were significantly associated with insulin resistance while other markers of adiposity (percent body fat, BMI, waist-to-thigh ratio) were not.[55]

Interestingly, intramyocellular lipid (IMCL) is also elevated in athletes but does not contribute to insulin resistance in this population.[56] Athletes posses a greater oxidative capacity for IMCL, which appears to be why this population is not insulin resistant.[56] Additionally, toxid lipid intermediates associated with IMCL in obesity, such as diacylglycerols (DAGs) and ceramides, are thought to contribute to insulin resistance in this population.[57] In a group of overweight and obese older adults, Dubé et al. demonstrated that following a 16-week aerobic exercise intervention, changes in insulin sensitivity were associated with ceramide content but not IMCL.[58] Therefore it is thought that the buildup of toxic lipid intermediates is responsible for insulin resistance in obese individuals.

Fat deposition in the liver also has a particularly strong relationship with insulin resistance. Visceral adipose tissue is associated hepatic lipid accumulation (IHL), and NAFLD is prevalent among obese individuals.[59] It is thought that lipolytic activity in visceral adipose tissue leads to increased delivery of FFA via portal circulation.[60] Kelley et al. reported that elevated fat content in the liver was significantly associated with severity of insulin resistance among obese diabetics. In this group, individuals with the highest lipid accumulation in the liver had significantly lower insulin-stimulated glucose uptake during a euglycemic insulin clamp .[59] IHL may also increase insulin resistance in other insulin sensitive tissues, including the liver, adipose tissue and skeletal muscle. In type 2 diabetics, both hepatic and adipocyte insulin resistance are significantly associated with elevated IHL.[61] Additionally, Korenlbat et al.

reported that IHL amount was directly correlated with impaired insulin action in the liver, adipose tissue, and skeletal muscle in a group of nondiabetic, obse adults.[62]

2.2.3 Adipocyte dysfunction and inflammation

In obesity, adiopocytes undergo both hypertrophy and hyperplasia in order to store excess fatty acids. Adipocyte enlargement triggers the unfolded protein response (UPR) in the endoplasmic reticulum, causing an inflammatory response and macrophage activation.[6] These processes result in adipocyte dysfunction and production of reactive oxygen species (ROS), adipokines and inflammatory mediators, which are all associated with insulin resistance.[3] Obesity is accompanied by upregulation of inflammatory pathways such as c-*jun* N-terminal kinase (JNK) and nuclear factor κB (NFκB) signaling, causing overproduction of proinflammatory cytokines.[52]

The proinflammatory cytokine tumor necrosis factor-α (TNF-α) appears to play a particularly important role in obesity-induced inflammation and insulin resistance. *In vitro* studies have demonstrated that TNF-α activates the JNK pathway and inhibits insulin receptor activation. This cascade of events results in insulin resistance by reducing the cellular response to circulating insulin.[63] In addition, TNF-α has been reported to independently predict rates of insulin-stimulated glucose uptake in a group of normal glucose tolerant and impaired glucose tolerant adults with a range of BMI classifications.[64]

Obesity contributes to insulin resistance, hyperinsulinemia and hyperglycemia by several potential mechanisms. Elevated FFA acid levels reduce insulin-stimulated glucose uptake and compromise normal fuel utilization in insulin resistant tissues. High FFA levels also lead to fat deposition in skeletal muscle and liver causing these tissues to become insulin resistant. Finally, adipocyte hypertrophy and hyperplasia initiate the upregulation of inflammatory pathways,

resulting in secretion of inflammatory mediators and adipokines that compromise insulin sensitivity. Therefore, hyperinsulinemia resulting from insulin resistance often accompanies obesity, putting this population at risk for many negative health consequences.

2.3 HEALTH CONSEQUENCES OF HYPERINSULINEMIA

While the etiology of insulin resistance is complex, there are several obesity-mediated factors that contribute to the development of insulin resistance, hyperinsulinemia and hyperglycemia. The initial decrease in insulin sensitivity is compensated by β-cells increasing insulin secretion, placing obese individuals in a state of hyperinsulinemia. While insulin resistance may eventually progress to diabetes, there are additional health consequences associated with chronic hyperinsulinemia. These health conditions include coronary artery disease, hypertension, dyslipidemia, NAFLD, PCOS, and impaired weight management efforts.

Over 20 years ago, Reaven identified hyperinsulinemia and insulin resistance as a central risk factor for coronary artery disease, hypertension, and dyslipidemia.[5] This clustering of risk factors associated with cardiometabolic disease is termed “metabolic syndrome,” and insulin resistance is considered the risk factor linking these conditions. Since Reaven initially identified the importance of hyperinsulinemia and insulin resistance, results from additional studies have supported the role of hyperinsulinemia in the development of cardiometabolic and other chronic disease.

2.3.1 Insulin and chronic disease

Several studies have observed a relationship between hyperinsulinemia and risk for chronic disease. For example, Zavaroni et al. reported that elevated insulin levels following a 75-gram OGTT were associated with increased incidence of diabetes, hypertension, and coronary heart disease. [65] This prospective study examined 647 healthy factory workers over a period of 12-15 years. The incidence of disease was significantly higher for those in the highest quartile for insulin level two hours following the glucose load. Among individuals in the highest insulin quartile, risk of diabetes was eight times higher, risk of hypertension was twice as high, and risk of coronary heart disease was three times greater compared to individuals in the lower three quartiles. Similarly, Facchini et al. explored the effect of insulin on a variety of chronic conditions, and reported that insulin resistance significantly predicted risk for "age-related" disease in 208 healthy, nonobese subjects. In this study "age-related disease" included coronary heart disease, cerebrovascular disease, hypertension, diabetes, and cancer. [66] Insulin resistance was evaluated using steady-state plasma glucose concentrations during a continuous infusion of insulin and glucose, and subjects were divided into tertiles based on insulin sensitivity. Over a mean follow-up period of 6.7 years, the tertile with the highest insulin sensitivity did not develop any age-related disease, while development of disease was significantly higher in tertile with the lowest insulin sensitivity. Collectively these data suggest that insulin is associated with the development of several chronic diseases, including heart disease, hypertension, diabetes and cancer.

2.3.2 Insulin and coronary artery disease

The relationship between insulin and cardiovascular disease has been examined extensively. Nondiabetic patients with diagnosed coronary artery disease are reported to have both higher fasting insulin levels during an OGTT and greater insulin-stimulated glucose uptake during a euglycemic insulin clamp when compared to healthy subjects without coronary artery disease.[67] Fasting insulin is also associated with increased risk of cardiovascular disease. In a group of nondiabetic men, Rubins et al. observed a 31% increased risk of a major CVD event among those in the highest quartile for fasting plasma insulin.[11] Similarly, Després et al. reported that hyperinsulinemia was an independent risk factor for ischemic heart disease in a group of men age 35-64 who were free from heart disease at baseline.[68] In this case-control study, fasting insulin was significantly associated with risk of ischemic heart disease; fasting insulin was 18% higher in the cases compared to controls at the end of a 12 year follow-up, and this association was independent of age, BMI, smoking status, lipids and alcohol use.

Hyperinsulinemia may also contribute to development of more complex atherosclerotic lesions. In non-diabetic and non-obese patients diagnosed with cardiovascular disease, fasting insulin and insulin response to an OGTT is associated with development of more complex atherosclerotic lesions.[12] The escalated progression of atherosclerosis is one potential mechanism explaining the relationship between hyperinsulinemia and cardiovascular disease.

While studies prior research demonstrates an association between fasting plasma insulin and heart disease, an even stronger relationship appears to exist between measures of insulin resistance and insulin response to a glucose load. In a cohort from the Framingham Offspring Study, insulin resistance measured by the homeostasis model assessment formula (HOMA-IR) and the insulin sensitivity index ($ISI_{0,\ 120}$) was significantly related to incident cardiovascular

disease over a median 6.7 year follow up period.[69] Insulin levels two-hours following an OGTT may also predict cardiovascular disease. In the San Antonio Heart Study, nondiabetic Mexican Americans and Non-Hispanic whites were followed for an average of 7.5 years. Increasing HOMA-IR scores were related to elevated risk of cardiovascular events, and this relationship remained significant after adjusting for other risk factors.[10] Additionally, a meta-analysis examining 19 prospective studies concluded that there is a significant association between insulin and risk for coronary heart disease.[70] The authors determined that the odds ratio for coronary heart disease was 1.35 with elevated non-fasting insulin, while the association between fasting insulin and heart disease risk was not significant. Therefore, examining the insulin response to a glucose challenge may be more indicative of risk than only measuring fasting insulin.

2.3.3 Insulin and cardiovascular disease risk factors

The connection between insulin and cardiovascular risk factors may be related to presence of risk factors. In particular, hyperinsulinemia and insulin resistance are associated with dyslipidemia and hypertension. Hyperinsulinemia is associated with low HDL-cholesterol and high plasma triglyceride concentrations,[13] as well as increased concentrations of apolipoprotein B (apoB)[71] and small dense LDL particles.[72-73] In a group of non-diabetic adults from the San Antonio Heart Study, fasting insulin and insulin during an OGTT were positively correlated with fasting triglyceride levels, and negatively correlated with HDL cholesterol and LDL particle size.[73] The inverse relationship between hyperinsulinemia and LDL particle size remained significant after adjusting for age, gender, BMI, waist-to-hip ratio and levels of triglycerides, HDL cholesterol and fasting plasma glucose. Garvey et al. reported similar results using insulin-stimulated

glucose disposal during a euglycemic clamp as a measure of insulin resistance.[72] In both diabetic and non-diabetic patients, LDL particle size decreased as insulin resistance increased, and this relationship was significant after adjusting for age, sex, race and BMI. In addition, Veerkamp et al. reported that subjects with familial combined hyperlipidemia (FCH) were significantly more insulin resistant as measured by HOMA, compared to normolipidemic relatives.[71] This relationship remained significant after adjusting for age, sex, and BMI. Subjects with FCH also had greater apoB levels and greater presence of small dense LDL with increasing levels of insulin resistance.

There also appears to be an association between insulin and development of hypertension. In an early prospective study, Skarfors et al. observed that fasting insulin and insulin response to an intravenous glucose tolerance test (IVGTT) significantly predicted development of hypertension in a group of 1796 Swedish men. In men who were normotensive at baseline, fasting insulin was 11.4% higher in men who developed hypertension over an average 10.2 year follow-up period. Serum insulin 60-minutes following administration of glucose during an IVGTT was also 23.4% higher in the men who developed hypertension. In a more recent cross-sectional study, approximately 50% of hypertensive adults were reported to be insulin resistant as measured by a modified insulin suppression test.[74]

2.3.4 Insulin and nonalcoholic fatty liver disease

NAFLD is another condition associated with hyperinsulinemia and insulin resistance. Patients with NAFLD are more insulin resistant compared to individuals without liver disease. Marchesini et al. reported that fasting insulin and average insulin during an OGTT were significantly higher in NAFLD patients compared to age and sex-matched controls.[75] Insulin

resistance was also the strongest predictor of NAFLD with an odds ratio of 15 (95% CI: 3.0-70) for each percent increase in HOMA-IR. A recent prospective study by Rhee et al. suggests that elevated fasting insulin might predict development of NAFLD.[15] In this study, fasting insulin in a group of non diabetic men and women of various BMI classifications was measured at baseline and again 5 years later. Subjects in the highest quartile for fasting insulin at baseline were at a significantly higher risk for developing NAFLD after five years, demonstrating that hyperinsulinemia may play a role in the pathogenesis of NAFLD.

2.3.5 Insulin and polycystic ovarian syndrome

Hyperinsulinemia and insulin resistance also contribute to the development of PCOS.[76] Burghen et al. first observed the association between insulin resistance and PCOS in an early cross-sectional study.[77] In this study women with PCOS had higher fasting insulin levels and a greater insulin and glucose response during a three-hour OGTT compared to obese controls. Insulin resistance appears to be independent of obesity in PCOS patients. Dobrjansky et al. reported impaired insulin-stimulated glucose uptake during an insulin clamp in women with PCOS, and adjusting for obesity and total adiposity did not change this relationship.[78]

2.3.6 Insulin and weight gain

In addition to increased risk for the above health conditions, elevated insulin levels may contribute to weight gain or negatively impact weight management efforts. The role of insulin is to facilitate glucose uptake for use and storage in tissues; thus, high levels may promote weight gain. For example, diabetics treated with insulin therapy often experience weight gain.[79-80]

Several prospective studies have also observed an association between insulin levels and weight gain over time. In a group of adults predisposed to diabetes, insulin levels following an OGTT predicted weight gain during an average 16.7 year follow-up period.[17] Insulin may also predict weight gain in children. In a group of Pima Indian children, fasting plasma insulin at baseline was significantly correlated with rates of weight gain and change in thickness of triceps skinfold thickness over a mean 9.3-year follow-up period.[18] This relationship remained significant after adjusting for age, sex, initial weight and change in height.

2.4 EFFECT OF EXERCISE ON INSULIN AND GLUCOSE

Due to the health consequences associated with insulin resistance, it is important to further explore therapeutic methods to decrease circulating insulin and glucose levels. Chronic exercise training is one such therapy which decreases insulin and glucose levels. Acute exercise also affects insulin sensitivity, and a single bout of moderate-intensity aerobic exercise is associated with decreases in circulating glucose and insulin, and improvements in insulin stimulated glucose uptake.

2.4.1 Effect of acute exercise on plasma insulin and glucose

Decreased plasma insulin and glucose have been observed following a single exercise session in healthy, obese and diabetic populations. Although insulin and glucose are not normally elevated in a healthy population, acute exercise still decreases these measures immediately post-exercise.

For example, a recent study reported a 5% decrease in glucose and a 10% decrease in insulin in healthy adults following 60-120 minutes of aerobic exercise at 60% VO_{2max}.[19]

The effect of acute exercise on plasma insulin and glucose has also been evaluated in obese and diabetic populations. These two groups are characterized by hyperinsulemia, insulin resistance, and potentially hyperglycemia. In an early study, Minuk et al. examined changes in fasting insulin and glucose following 45 minutes of aerobic exercise at 60% VO_{2max} in obese diabetics and obese non-diabetics.[81] Insulin decreased significantly from 0.90±0.15 ng/ml to a nadir of 0.65±0.09 ng/ml during exercise in the obese nondiabetic group, but no significant changes were observed in the obese diabetic group. Fasting plasma glucose was elevated in the diabetics compared to the nondiabetics, but exercise did not significantly change glucose in either group. Conversely, Martin et al. reported a significant decrease in glucose from 10.0±0.9 mmol/L to 9.0±0.9mmol/L in diabetic subjects following 40 minutes of exercise at 60% VO_{2max}.[82] However, no change was observed in a lean, nondiabetic control group and insulin did not significantly decrease in either group.

While results examining changes in fasting measures are conflicting, observations in studies measuring postprandial changes in insulin and glucose are more consistent. Poirier et al. observed that sixty minutes of exercise at 60% VO_{2max} decreased postprandial glucose from 8.2±0.4 mmol/L to 6.0±0.2 mmol/L in a group of 19 men with type 2 diabetes and various BMI classifications.[83] Larsen et al. also reported a decrease in postprandial glucose and insulin following both moderate[22] and high-intensity exercise in diabetic men.[84] Both a single session of moderate intensity exercise for 45 minutes and four sessions of high intensity exercise separated by six minutes of rest significantly decreased plasma insulin and glucose following a breakfast meal. Moderate intensity exercise decreased the area under the curve (AUC) for

glucose and insulin during an OGTT by 52% and 34%, respectively.[22] Following the high intensity exercise, plasma glucose was decreased from 12.6±0.7 mmol/L to 11.4±0.8 mmol/L and insulin decreased from 144±27 pmol/L to 93±21 pmol/L.[84]

Recently, van Dijk et al. reported a significant reduction in 24-hour average blood glucose values following a single aerobic exercise session.[85] In this trial subjects with either impaired glucose tolerance or type 2 diabetes participated in 45 minutes of stationary cycling at 50% of their maximum workload capacity at the beginning of a 24-hour observation period. Subjects were fed a standardized diet preceding and throughout the observation period. All exercise sessions were performed 2.5 hours following breakfast, and blood glucose was measured using continuous glucose monitoring. Average blood glucose was 7.4±0.2 mmol/L in the impaired glucose tolerance group during the control condition, and decreased to 6.8±0.2 mmol/L when subjects exercised. Similarly, average blood glucose was 9.6±0.5 and 9.2±0.7 mmol/L during the control session in type 2 diabetics treated with oral medications and insulin, respectively; these values decreased to 8.6±0.5 and 8.5±0.5 mmol/L with the exercise session. Therefore, based on the current evidence it is possible that acute exercise may have a greater effect on insulin and glucose when performed in a postprandial state compared to a fasting state.

2.4.2 Effect of acute exercise on insulin-stimulated glucose uptake

Insulin-stimulated glucose uptake is also improved following exercise in an obese population. In an early study, Burstein et al. reported improved insulin action measured using a three-stage euglycemic clamp immediately following exercise in obese and diabetic individuals. Subjects exercised for 60 minutes at a heart rate of 150-160 beats/minute. Compared to a lean control group, insulin was elevated and glucose disposal was decreased in obese and diabetic

individuals. Following the exercise session circulating insulin decreased and insulin-stimulated glucose uptake increased in the obese, nondiabetic group, but not the lean control group. Exercise increased the maximal clearance rate of glucose at the highest serum insulin concentration of the three stage insulin clamp by 124% in the obese diabetics and 134% in the obese group. Cusi et al. observed similar results during a euglycemic insulin clamp performed 24 hours following an acute exercise session. Lean controls, obese nondiabetics, and diabetics each participated in 60 minutes of aerobic exercise at 65% VO_{2max}. Insulin-stimulated glucose disposal was improved in the obese non-diabetics, but not the diabetics. In the obese nondiabetics, glucose disposal during the 90-120 min period of an insulin clamp improved from 3.65±0.74 to 4.32±0.85 mg/kg/min following the 60-minute exercise session. These findings suggest that exercise may be an important mechanism to decrease insulin response and improve insulin action in an obese population.

2.4.3 Time course for changes in insulin following acute exercise

While acute exercise improves insulin sensitivity following exercise, this effect is transient. A single bout of exercise has been shown to increase insulin sensitivity for a period of two to 72 hours following the exercise session.[23-25] King et al. examined the time course of improved insulin sensitivity in a group of healthy, trained men and women following five consecutive days of aerobic exercise training. An OGTT was performed on days one, three, five and seven following the final exercise session. On days one and three the insulin AUC in response to a glucose load was significantly lower. By days five and seven, no differences in the insulin AUC after an OGTT were observed compared to baseline. Similar responses were reported in a cohort from the HERITAGE family study. Following 20 weeks of aerobic exercise training, fasting

insulin significantly decreased by 8% in the 24 hours following an exercise bout, but returned to baseline 72 hours post-exercise.[25] Thus, insulin sensitivity following exercise is transient, even in a trained population. While current research suggests the effect of exercise does not last longer than 72 hours, there is currently limited evidence regarding the specific time course of changes in insulin, particularly in an obese population.

2.4.4 Chronic effect of short bouts of exercise on insulin and glucose

Current physical activity recommendations indicate that activity can be accumulated in bouts lasting a minimum of 10 minutes.[26-28] Research suggests that accumulating physical activity in 10-minute bouts effectively increases cardiovascular fitness[29-36] and promotes weight loss.[29-31] These improvements are equivalent or superior to changes following continuous exercise training. For example, Murphy et al. observed significant improvements in fitness between groups exercising once per day for 30 minutes and those performing three 10-minute bouts per day. However, only the group performing short bouts significantly decreased body mass and waist circumference. While fitness and are improved by accumulating short bouts of activity, the effect of these programs on other health outcomes such as insulin and glucose are not yet fully understood.

Although there is limited research on the effectiveness of short bouts for decreasing insulin and glucose and improving insulin resistance, current exercise recommendations for obese[28] and diabetic adults[24] suggest performing short bouts to meet daily activity requirements. It is possible that performing activity in short bouts improves insulin and glucose as effectively as exercising for one continuous session. However, few studies have explored this question and current findings are equivocal.

In one study, Donnelly et al. examined the effect of 18 months of intermittent versus continuous exercise training on metabolic variables, including insulin and glucose in overweight or obese females.[32] The continuous group performed 30 minutes of exercise at 60-75% VO_{2max} three times a week. The intermittent group exercised twice a day, five days per week at 50-65% heart rate reserve (HRR). 18 months of training significantly decreased fasting insulin in the intermittent exercise group only, while insulin AUC during a two-hour OGTT decreased in both groups. Fasting insulin decreased from 15.81±14.95 μU/mL to 11.90±9.38 μU/mL. There were no changes in fasting glucose or glucose during the OGTT in either group following exercise training. Conversely, Eriksen et al. reported the opposite effect for insulin and glucose in a group of 18 overweight or obese diabetic adults.[37] Subjects performed 30 minutes of aerobic exercise training, six days per week at 60-65% VO_{2max}. Exercise was performed either in one continuous 30-minute bout or three 10-minute bouts throughout the day. Following five weeks of training, fasting glucose decreased by 0.5 mmol/L and glucose AUC during an OGTT decreased by 7.5% in the intermittent exercise training group only. No changes were seen in insulin measures for either exercise group.

While the changes observed in insulin and glucose following intermittent exercise training are inconsistent, these differences may be related to the population studied. Donnelly et al. reported changes in insulin but not glucose in a group of overweight or obese females. However, glucose but not insulin was decreased in the diabetic subjects studied by Eriksen et al. It is possible that these conflicting findings are related to differences in insulin and glucose levels between populations based on the degree of insulin resistance present. As discussed previously, in the early stages of insulin resistance increased insulin secretion overcomes hyperglycemia and maintains normal glucose levels. As insulin resistance progresses to diabetes, insulin secretion is

impaired and hyperglycemia results. Therefore, an obese nondiabetic population as studied by Donnelly et al. may demonstrate larger changes in insulin with exercise, while blood glucose may be affected to a greater degree than insulin in diabetic subjects. Due to the limited data and differences observed between obese and diabetic adults, it is important to further explore the effect of exercise on changes in insulin and glucose in these populations.

2.4.5 Acute effect of short bouts of exercise on insulin and glucose

The literature regarding the acute effect of accumulating short bouts of exercise on changes in insulin and glucose is limited, and most studies have been performed in healthy adults. For example, Goto et al. examined the effect of either one continuous 60-minute exercise session compared to two 30-minute sessions separated by a 20 minute rest period in eight healthy, physically active men.[86] All exercise sessions were performed at 60% VO_{2max}. Plasma insulin and glucose were collected at 15-minute intervals during and after the exercise sessions. During the exercise sessions both insulin and glucose were significantly lower in the second half of the repeated exercise trial compared to the single exercise trial. During the second half of the exercise session insulin was significantly lower in the repeated exercise session (1.8±0.3μU/ml) compared to the continuous session (2.9±0.4μU/ml). In the repeated exercise trial, glucose decreased from 89±2 mg/dL to 83±1 mg/dL, during the second half of the exercise session. However, there were no differences between exercise sessions observed in recovery for insulin or glucose.

Changes in insulin and glucose following short bouts of exercise have also been observed in healthy subjects when measured the morning after an exercise session. In an early study, Gill et al. had 18 healthy males run at 60% of VO_{2max} for a single 90-minute session, three 30-minute

sessions spread throughout the day, and a control session with no exercise.[38] The next morning, subjects were given a high-fat meal (1.2 g fat, 1.2 g carbohydrate, 70 kJ/kg body weight) and blood samples were obtained hourly for a six hours. No differences in fasting insulin were observed between the two exercise treatments, while insulin AUC was lower following short bouts compared to the control condition. There were no changes reported in fasting glucose or glucose AUC in the continuous session. Miyashita et al. recently reported similar results.[39] In this study, ten healthy, recreationally active men participated in either three 10-minute sessions with 30 minutes of rest in between, or a single 30-minute session. All exercise sessions were performed at 70% VO_{2max}. The next day subjects consumed a mixed meal (56% fat, 33% carbohydrate, 11% protein, 46 kJ/kg body weight) for breakfast and lunch. The meals were consumed three hours apart and blood was sampled each hour for a 7-hour period. In concordance with the results from Gill et al.,[38] postprandial plasma insulin AUC was significantly lower following the meals after performing multiple short bouts of activity compared to the continuous exercise session. There were no changes in fasting insulin, glucose, or glucose response to the test meals in any of the experimental conditions.

While multiple short bouts of exercise appear to have an acute effect on postprandial insulin levels in a healthy population, there is limited data in obese or diabetic populations. A recent study explored the effect of 60 minutes of aerobic exercise performed on a single day, compared to two 30-minute sessions performed on consecutive days in a group of male type 2 diabetics.[87] Each subject participated in a control condition, 30 minutes of exercise performed on two consecutive days, and 60 minutes of exercise performed in a single bout. All exercise sessions consisted of stationary cycling at a 50% of maximum workload, and were performed 1.5 hours following a breakfast meal. Subjects were fed a standardized diet during the 48-hour

observation period and blood glucose was measured using continuous glucose monitoring. Additional blood draws were performed before breakfast in the fasting state and 2.5 hours after breakfast. Both 30 and 60 minutes of exercise significantly lowered plasma insulin and glucose in a dose-dependent manner 2.5 hour following breakfast. However, there was no difference in fasting insulin or glucose between the 60-minute and a 30-minute session the following morning. At the 2.5 hour post-breakfast blood draw on the second day, postprandial glucose was significantly lower than the control after performing the single 60-minute session the previous day and the second 30-minute session that morning. However, the 30-minute exercise performed that morning produced a significantly greater effect. Postprandial insulin was only significantly decreased when 30 minutes had been performed that morning, and there was no effect from the 60-minute session performed the prior day. These results suggest that performing shorter duration exercise on multiple days may be as effective as one day of continuous exercise for controlling insulin and glucose in type 2 diabetics, as long as energy expenditure is equivalent.

Currently, only one study has examined the effect of accumulating activity in short bouts in nondiabetic adults. Murphy et al. recruited 10 apparently healthy men and women with BMI < 35 kg/m^2; BMI classification ranged from 23.9 kg/m^2 to 34.5 kg/m^2. Subjects participated in one 30-minute exercise session before breakfast, or a 10-minute session before breakfast, lunch and dinner. All exercise sessions were performed at 60% VO_{2max}. Blood samples were collected throughout the entire day, and there were no changes in insulin or glucose observed at any timepoint in either session. This is currently the only study examine look at the effect of short bouts of exercise on insulin and glucose in obese subjects, with BMI ranging from normal weight to obese, making these findings difficult to generalize to an obese population.

2.4.6 Gaps in the literature

Several studies have examined the effect of exercise on changes in insulin and glucose. However, there are methodological differences in the measurement of insulin and glucose (fasting, response to an OGTT, euglycemic insulin clamp) between these studies, making it difficult to interpret the results. Additionally, the populations included in the current research include healthy, obese and diabetic individuals. These groups vary with regards to severity of insulin resistance, and subsequent response to insulin and glucose. Therefore, it is not possible to generalize the findings between the groups.

The literature is limited regarding the effectiveness of short bouts of activity for improving plasma insulin and glucose in an obese population. Data from exercise training studies suggest that accumulating short bouts of activity may result in lower insulin response to a glucose load in an obese population. In normal weight and diabetic populations, exercise appears to decrease postprandial insulin. In an obese population, exercise before a meal may not significantly decrease insulin and glucose, but the effect of exercise on insulin and glucose when performed in the postprandial state is currently unknown in this population. Therefore, the purpose of this study is to examine the postprandial effect of insulin and glucose in response to a 10-minute and a 30-minute exercise session in obese adults.

3.0 METHODS

It is currently estimated that approximately one-third of the U.S. adult population is obese.[1] Obesity is associated with increased risk of multiple chronic diseases.[2] It has been suggested that hyperinsulinemia accompanying insulin resistance is the central risk factors connecting obesity to the increased development of health complictations.[5, 13]

An acute exercise session is associated with decreases in plasma insulin and glucose. Current exercise prescription guidelines suggest that exercise can be performed in multiple short bouts, but it is unknown how shorter exercise sessions affect insulin and glucose compared to a single, longer exercise session. The aim of this study was to examine the effect of a 10-minute bout of exercise compared to a 30-minute bout on changes in postprandial insulin and glucose. This was done to better explain if performing short bouts of exercise may have an equal or better effect on decreasing insulin and glucose in an obese population.

3.1 SUBJECTS

A total of 11 apparently healthy men and women between the ages of 35-55 were recruited to participate in this study. Subjects were Class I, Class II, or Class III Obese according to BMI classification (30.0-<45.0 kg/m^2), and were sedentary defined as exercising at a moderate-intensity less than 60 minutes per week over the last six months.

Additional exclusionary criteria for this study included:

1) Previous diagnosis of cancer, heart disease, Type I or Type II diabetes, or polycystic ovarian syndrome. Individuals with these conditions require medical clearance that was beyond the proposed scope of this study.[28] Additionally, the response of glucose and insulin in individuals with diabetes and polycystic ovarian syndrome may be altered and may affect measures of the primary outcomes (i.e., insulin and glucose).
2) Taking prescription or over-the-counter medications that affects glucose metabolism, insulin, blood pressure or heart rate. Medications that alter glucose metabolism or insulin (such as thiazolidinediones, oral glucocorticoids, nicotinic acid, and oral steroids) may alter how these variables respond to exercise. Subjects were asked to perform a submaximal graded exercise test to 85% of age-predicted maximal heart rate, and medications altering heart rate affect the ability to determine termination heart rate during this test. Additionally, the intensity of exercise during testing sessions was determined using heart rate, and medications altering heart rate would affect the ability to determine appropriate exercise intensity.
3) Presence of any condition that may limit one's ability to walk for exercise (e.g., orthopedic limitations or severe arthritis). Subjects were required to walk for exercise to complete the sub maximal graded exercise test and subsequent testing sessions, and any orthopedic limitations would limit the ability of these individuals to complete these components of this study.
4) Currently participating in a weight loss program or reporting significant weight loss (>3.0% of body weight) in the past month. Changes in weight have been shown to

influence glucose metabolism and insulin, and subjects were asked to maintain their current body weight during their participation in the study.[88-90]

5) Women who were pregnant at any point during the study. Vigorous exercise is not recommended for women who are pregnant, which would limit the ability of these women to participate in the graded exercise test in this study.[28] Pregnancy also affects glucose and insulin levels,[91-92] which would influence the main outcome measures of the study.

6) A resting systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg or currently taking prescription medication to control blood pressure. Blood pressure at these levels or currently taking medication to control blood pressure contraindicates participation in a submaximal graded exercise test without the supervision of a physician.[28] Therefore, blood pressure at these levels posed a safety concern in this study.

7) Premenopausal women who reported irregular menstrual cycles (<25 days or >35 days between cycles). Hormonal variations during the menstrual cycle affect glucose and insulin levels,[93-94] thus all premenopausal female subjects were tested during days 1-14 of the menstrual cycle. If premenopausal women do not have regular menstrual cycles it impaires the ability to schedule them so that testing sessions could occur during this period of time.

8) Report consuming more than a moderate amount of alcohol (>7 drinks per week for females and >14 drinks per week for males)[95] Alcohol consumption affects blood glucose levels, and excess consumption would affect glucose levels during the testing sessions.

3.2 RECRUITMENT AND SCREENING PROCEDURES

Subjects were recruited through local flier postings and Craigslist advertisements. Additionally, letters were mailed to individuals meeting eligibility requirements registered in the Obesity and Nutrition Research Center (ONRC) database. The University of Pittsburgh Institutional Review Board (IRB) approved all recruitment methods and materials. Interested individuals were instructed to call the University of Pittsburgh Physical Activity and Weight Management Research Center (PAWMRC). Trained staff and graduate students at the PAWMRC read a description of this study and delivered a brief phone screening to participants who provided verbal consent. Screening information included questions regarding demographic background, physical health and medical history to determine initial eligibility.

Individuals who were found to be eligible following the phone screening were invited to attend an orientation session conducted by the Principle Investigator (PI). During this orientation the PI reviewed the study protocol and gave individuals an opportunity to answer any questions before signing an informed consent document. Subjects also completed a Physical Activity Readiness Questionnaire (PAR-Q)[96] to ensure that participation in exercise was not contraindicated. Eligible subjects then underwent an initial assessment and three experimental sessions as described below. The IRB at the University of Pittsburgh approved all study procedures.

3.3 ASSESSMENT PROCEDURES

3.3.1 Height

Height was measured using a wall-mounted stadiometer (Perspective Enterprises; Portage MI). The subject removed their shoes and stood erect on the floor with his/her back parallel to the vertical mounted measure scale, looking straight ahead. The subject was instructed to stand as straight as possible with feet flat on the floor. The horizontal measuring block was brought down snugly, but not tightly, on top of the head. Height was recorded to the nearest 0.1 cm.

3.3.2 Body weight and body mass index

Body weight was measured using a Tanita WB-110A digital scale (Tanita Corporation; Arlington Heights, II). Measurements were made with subjects wearing a lightweight hospital gown with shoes removed. Weight was recorded to the nearest 0.1kg. BMI was computed based on measurements of weight and height and was calculated as body weight in kilograms divided by square height in meters (kg/m^2).

3.3.3 Resting seated heart rate and blood pressure

Resting blood pressure and heart rate was assessed using a DINAMAP V100 (GE Healthcare) automated blood pressure system. Cuff size was determined by arm circumference. Each of the following cuff sizes was available: Adult, Large Adult, Thigh and Long Large Adult. A Gulick Tape Measure was used for measurement of the participant's arm circumference. Arm

measurement was performed on the lateral aspect of the arm at the midpoint between the acromion process to the olecranon process. The cuff size was determined from the arm circumference measurement according to the following chart:

Arm Circumference	Cuff Size
17.0 to <24.0 cm	Small Adult
24.0 to <33.0 cm	Adult
33.0 to <41.0 cm	Large Adult
>41.0 cm	Thigh or Large Adult Long*

*(If a participant's upper arm circumference indicated use of the thigh cuff, but the arm was too short for the cuff, or the cuff did not remain secured when inflated, the Large Adult Long arm cuff was used.)

Blood pressure was measured with subjects seated and both feet flat on the floor. Subjects rested quietly for a five-minute period prior to the first measurement. A second measurement was taken following a minimum 60-second waiting period. A third measurement was obtained if the two systolic blood pressures differ by ≥10 mm Hg or the diastolic blood pressures differed by ≥6 mm Hg.

The Dinamap automated blood pressure system also provided a measure of heart rate. Thus, resting heart rate was measured simultaneously with the measures of resting blood pressure as described above.

3.3.4 Waist circumference

A Gulick Tape Measure was used for obtaining waist girth measurements. All measurements were taken with the subject in a lightweight hospital gown. Waist circumference was measured

horizontally at the iliac crest. To determine the level at which waist circumference was measured the examiner faced the subject and palpated the upper hip bone to locate the iliac crest. The subject then placed their fingertips directly above the iliac crest and the examiner placed the measuring tape around the abdomen directly below the fingertips. Duplicate measurements were taken at the end of a normal exhalation measurements were recorded to the nearest 0.1cm. A third measurement was taken if the two measures differed by $\geq$1.0 cm.

3.3.5 Graded exercise test

Cardiorespiratory fitness was evaluated using a submaximal graded exercise test utilizing a protocol developed for the Look AHEAD study.[97] The speed of the treadmill was kept constant at 3.0 mph (80.4 m/min) throughout the test. The initial grade of the treadmill was set to 0% and increased at 1.0% increments each minute until the subject reached 85% of age-predicted maximal heart rate, determined by the equation: 220-age. The metabolic cart (SensorMedics Vmax Metabolic Measuring Cart; SensorMedics; Yorba Linda, CA) was calibrated for air volume and gas analysis prior to each test. A certified Clinical Exercise Specialist as recognized by the American College of Sports Medicine conducted all graded exercise tests.

Prior to the test, subjects entered the testing room and were given a brief overview of the test protocol. The exercise specialist explained the Borg 15-point Rating of Perceived Exertion Scale.[98] PAWMRC staff then prepared the subject for the exercise test by cleaning the sites for each of the 12 leads with rubbing alcohol and placing electrodes over these sites. The electrodes were then attached to the leads for 12-lead EKG monitoring. The Exercise Specialist then obtained a seated resting EKG printout prior to beginning the test to assure that exercise was not contraindicated.

The subject was then fitted with the airflow mouthpiece and head support system before the test began. The speed of the treadmill was set at 3.0mph (80.4 m/min) and remained constant throughout the test. The initial grade was set to 0.0%. Heart rate during exercise testing was obtained at one-minute intervals and immediately upon termination of the exercise test using 12-lead ECG (Cardiosoft, SensorMedics; Yorba Linda, CA). Blood pressure was obtained during each even minute (2 min, 4, min, 6 min etc.) and immediately upon termination of the exercise test. Rating of perceived exertion was assessed immediately following blood pressure measurement and at the point of test termination. Expired gas volumes and concentrations were measured continuously during the test to determine oxygen uptake and substrate utilization throughout the test. The test was terminated when the subject reached 85% of their maximum heart rate or following any signs or symptoms indicative of test termination as described by the American College of Sports Medicine. Following test termination, Subjects performed a three minute active recovery at 0% grade and 2.0-2.5% mph. During the active recovery the airflow mouthpiece and head support system was removed. Blood pressure was assessed during active recovery, and then the subject sat on a hospital bed to allow heart rate and blood pressure to return to resting values. A board certified cardiologist evaluated the results of all tests to ensure that exercise was not contraindicated. All testing staff and personnel were certified in cardiopulmonary resuscitation (CPR) and use of the Automated External Defibrillator (AED), and safety equipment was readily accessible in the testing room.

3.4 EXPERIMENTAL DESIGN

Eligible subjects reported to the PAWMRC for three separate experimental sessions following the initial assessment visit. These included a 10-minute exercise testing session (10-EX), a 30-minute exercise testing session (30-EX), and a sedentary testing session (REST). Subjects were randomized using a counterbalanced design to complete these sessions. All testing session occurred in the morning following a 12-hour fast. Since hormonal changes throughout the menstrual cycle affect insulin and glucose,[93-94] all premenopausal women were tested during days 1-14 of their menstrual cycle as determined by self-report. All three testing sessions were performed within a 31 day time period.

Prior to each experimental session subjects adhered to the following protocol: 1) refrained from exercise for 72 hours prior to the experimental session; 2) kept a detailed food record for one day prior to the experimental session; 3) avoided alcohol for 24 hours prior to the experimental session; 4) abstained from all food and drink other than water for 12 hours prior to the experimental session. Prior to the first experimental session subjects recorded all food and drink consumed during the previous day, and this record was photocopied and returned to subjects. Subjects were asked to replicate this food intake as closely as possible for the subsequent testing sessions to prevent differences in diet from influencing outcome measures. Compliance to these directions was verified via self-report prior to each experimental session.

3.5 EXPERIMENTAL SESSIONS

Subjects reported to the PAWMRC on the morning of each experimental session. Upon arrival the subject verified adherence to the protocol for pre-session directions as stated above. The subject was weighed, fitted with a Polar Heart Rate Monitor (for the 10-EX and 30-EX conditions), and all testing procedures were reviewed.

An initial blood draw to assess fasting glucose and insulin was then performed on each subject. Subjects were then provided a standardized breakfast containing 20% of their estimated energy requirement, composed of 55% carbohydrate, 30% fat and 15% protein (See Appendix M). This macronutrient distribution is within the range recommended by the U.S. Department of Agriculture and U.S. Department of Health and Human Service.[95] Additionally, exercise has been shown to reduce blood glucose following a breakfast meal containing this macronutrient distribution.[22] Estimated energy requirement was determined using the appropriate sex-specific Mifflin-St. Jeor equation to calculate resting energy expenditure and then multiplied by an activity factor of 1.2 for sedentary men or sedentary women.[99] Subjects were given 15 minutes to consume the breakfast meal. During the initial experimental session the time to consume the meal was recorded and then was replicated during subsequent experimental sessions.

Subjects remained in the laboratory for a 120-minute testing period following the breakfast meal. After consuming breakfast, subjects were asked to sit quietly for thirty minutes before another blood draw was performed. Immediately following this blood draw, subjects participated in either ten minutes of exercise (10-EX condition), thirty minutes of exercise (30-ex), or continued their resting period (REST condition). The flow of these experimental sessions is outlined in figure 1, and details are described below:

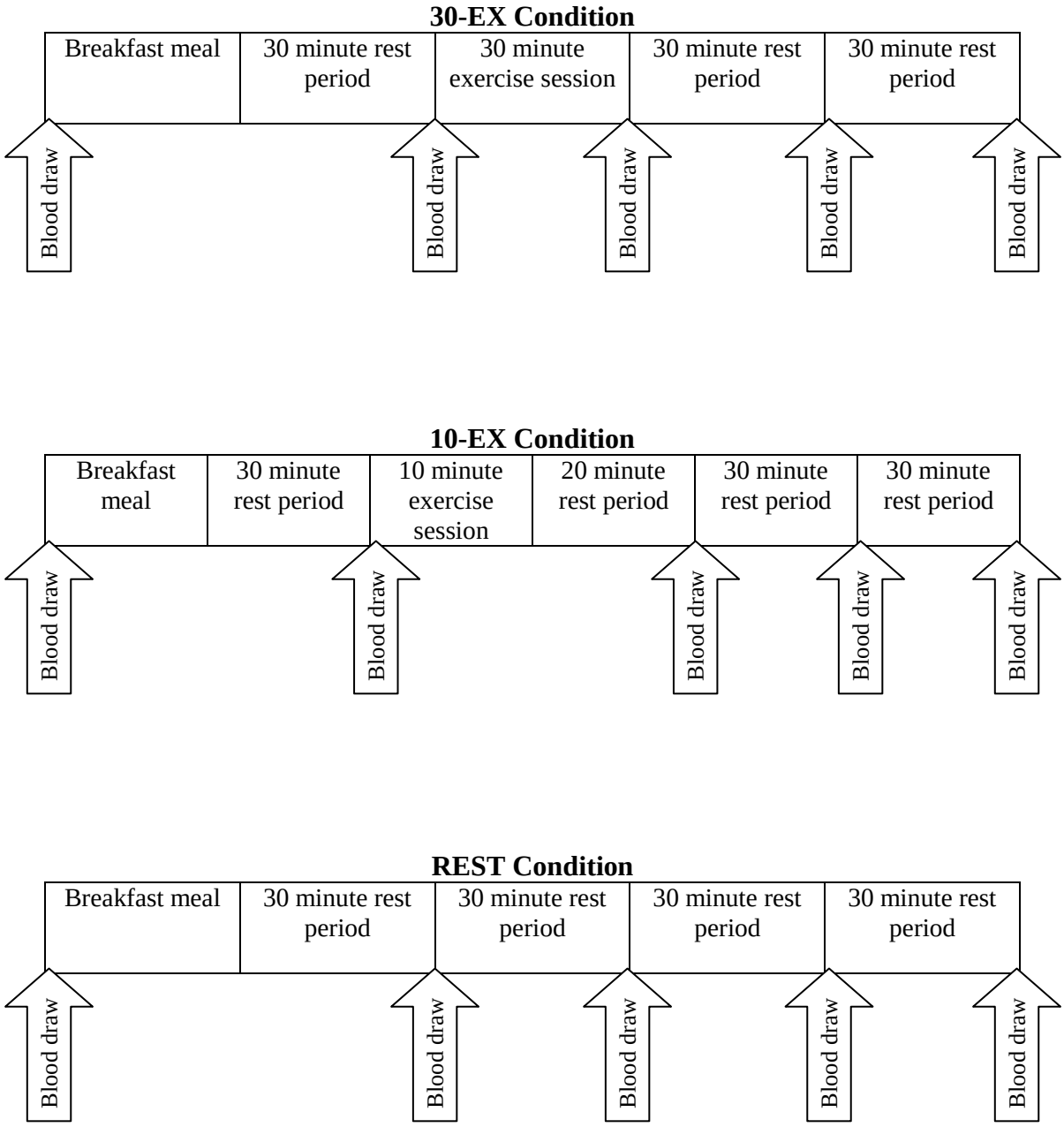


Figure 1: Flow of subjects through testing sessions

<u>10-EX Condition</u>: Subjects walked on a treadmill for 10 minutes at a speed of 3.0 mph and a grade that induced a heart rate between 70-75% of the subject's age-predicted maximal

heart rate. Grade was initially set using data available from the graded exercise test performed during the assessment visit. Heart rate was monitored during the last 5 seconds of each minute, and grade was adjusted if the subject's heart rate fell outside of the target heart rate range for two consecutive minutes using the following protocol: 1) if the heart rate was below the range of 70% to 75% of age-predicted maximal heart rate the grade was increased by 1%, 2) if the heart rate was above the range of 70% to 75% of age-predicted maximal heart rate the grade was decreased by 1%. If a grade of 0% elicited a heart rate greater than the target range the speed was decreased in 0.2 mph increments.

Exercise was terminated at the end of ten minutes of walking. Subjects then rested quietly for the remainder of the 120 minute testing period (80 minutes). During this time, they were permitted to watch a video or read magazines provided by the investigator. Subjects were also allowed to read materials or watch videos that were approved by the investigator. Subjects were allowed to consume water ad libitum throughout the entire experimental condition. Subjects underwent blood draws every 30 minutes during the testing period following breakfast consumption, occurring at 30, 60, 90 and 120 minutes.

30-EX Condition: Subjects walked on a treadmill for 30 minutes at a speed of 3.0 mph and a grade induced a heart rate between 70-75% of the subject's age-predicted maximal heart rate. Selection of initial grade and any alterations to grade and/or speed occurred in an identical manner as described above for the 10-EX condition. The only difference in the two exercise sessions was the length of time the subject walked on the treadmill. Exercise was terminated following 30 minutes of walking. Subjects then rested quietly for the remainder of the 120 minute testing period (60 minutes). During this time, they were permitted to watch a video or read magazines provided by the investigator. Subjects were also allowed to read materials or

watch videos that were approved by the investigator. Subjects were allowed to consume water ad libitum throughout the entire experimental condition. Subjects underwent blood draws every 30 minutes during the testing period following breakfast consumption, occurring at 30, 60, 90 and 120 minutes.

REST Condition: Subjects were instructed to rest quietly in a seated, upright position for the 120 minute testing session. During this time, they were permitted to watch a video or read magazines provided by the investigator. Subjects were also allowed to read materials or watch videos that were approved by the investigator. Every thirty minutes subjects underwent a blood draw, occurring at 30, 60, 90 and 120 minutes after the subject finished consuming the breakfast meal. Subjects were allowed to consume water ad libitum during the entire testing session.

3.6 PRIMARY OUTCOME MEASURES

Insulin and Glucose were analyzed at the Heinz Laboratory using the following procedures.

Insulin: Insulin was measured using an RIA procedure developed by Linco Research, Inc. Cross-reactivity of the antibody with human proinsulin is under 0.2%. Briefly, samples were mixed with ^{125}I-insulin and insulin antibody and then incubated at room temperature for 18 to 24 hours. The insulin-antibody complex was precipitated during a 20 minute incubation at 4 degrees C and subsequently sedimented by centrifugation for 15 min at 3,000g at 4 degrees C. Finally, the supernatant was decanted and the pellets were counted. Under these conditions the limit of sensitivity is 2 μU/ml and the response is linear up to 200 μU/ml. Standards, blanks,

quality controls and commercial serum control pools were run simultaneously with all samples. The coefficient of variation between runs is 8.2 +/- 0.7 (170)%. The intra assay CV% is 7.0 %.

Glucose: Serum glucose was quantitatively determined using an enzymic determination read at 340/380 nm, utilizing the coupled enzyme reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase. Using the Sigma Diagnostics glucose [HK] 20 reagent, serum was diluted 1:101. The mixture was then incubated for four minutes at 37°C and read at 340/380 nm using the Abbott VP Supersystem spectrophotometer. The increase in absorbance at 340/380 nm is directly proportional to the glucose concentration of the sample. The coefficient of variation between runs is 2.0%. The intra assay coefficient of variation is 1.6%.

3.7 STASTICAL ANALYSIS

Statistical analysis was performed using PASW software. Statistical significance was set at $p < 0.05$. Descriptive analyses were performed for subject's height, weight, BMI, blood pressure, waist circumference, $VO_{2peak,}$ and average fasting insulin and glucose.

To examine specific aims 1 and 2, separate one-way repeated measures analysis of variance (ANOVA) were performed on AUC for insulin and glucose across treatment conditions. AUC was calculated using the trapezoidal method. The assumption of normality was tested using the Shapiro-Wilk test and assumption of sphericity was tested using Mauchly's test. If the assumption of sphericity was not met the Greenhouse-Geisser was used. For variables that were not normally distributed the equivalent nonparametric tests were performed. When necessary, post hoc comparisons were made using the Bonferonni adjustment to determine which variables

were significantly different. A one-way repeated measures ANOVA was also performed on both insulin AUC above fasting AUC, and glucose AUC above fasting AUC.

As exploratory analyses, a one-way ANOVA was performed on each time point across the three conditions, and for the change in insulin and glucose from fasting at each time point. In addition, correlations between energy expenditure and insulin and glucose AUC were examined. Data for energy expenditure was not normally distributed and Spearman rank correlations were performed. Partial correlations adjusting for body weight and fitness were analyzed.

3.8 POWER ANALYSIS

The aim of this study was to determine the change in insulin and glucose following 10-minutes of aerobic exercise, 30-minutes of aerobic exercise, or during a resting control period. Previous research examining area under the curve for postprandial changes in insulin and glucose following 45 minutes of exercise report an effect size of r=0.85 for glucose and r=0.55 for insulin.[22] This study examined exercise of shorter duration (30 minutes and 10 minutes) and it was anticipated that a smaller effect size would be observed. Therefore it seemed reasonable to estimate a moderate effect size of r=0.50 for this study. Using power of 0.70 and alpha of 0.05, 25 subjects were needed to detect an effect size of 0.5.

4.0 RESULTS

The purpose of this study was to examine the postprandial effect of insulin and glucose in response to a 10-minute and a 30-minute exercise session in obese adults. This study utilized a randomized cross-over design and the results from the study are presented in the following sections.

4.1 SUBJECTS

A total of 21 obese men and women attended a study orientation and consented to participate in this study (Figure 2). Six individuals failed to either schedule or attend the assessment, and 4 were discovered to be ineligible at the assessment period. 11 obese men and women (mean BMI: 34.4 kg/m^2) completed the assessment and were eligible to initiate experimental sessions. However, two subjects did not complete the experimental sessions due to an inability to obtain blood during the initial blood draw and did not proceed further with the experimental sessions. Thus, data were collected on a total of 9 subjects (Figure 2). Descriptive statistics (mean ± standard deviation) are shown in Table 1.

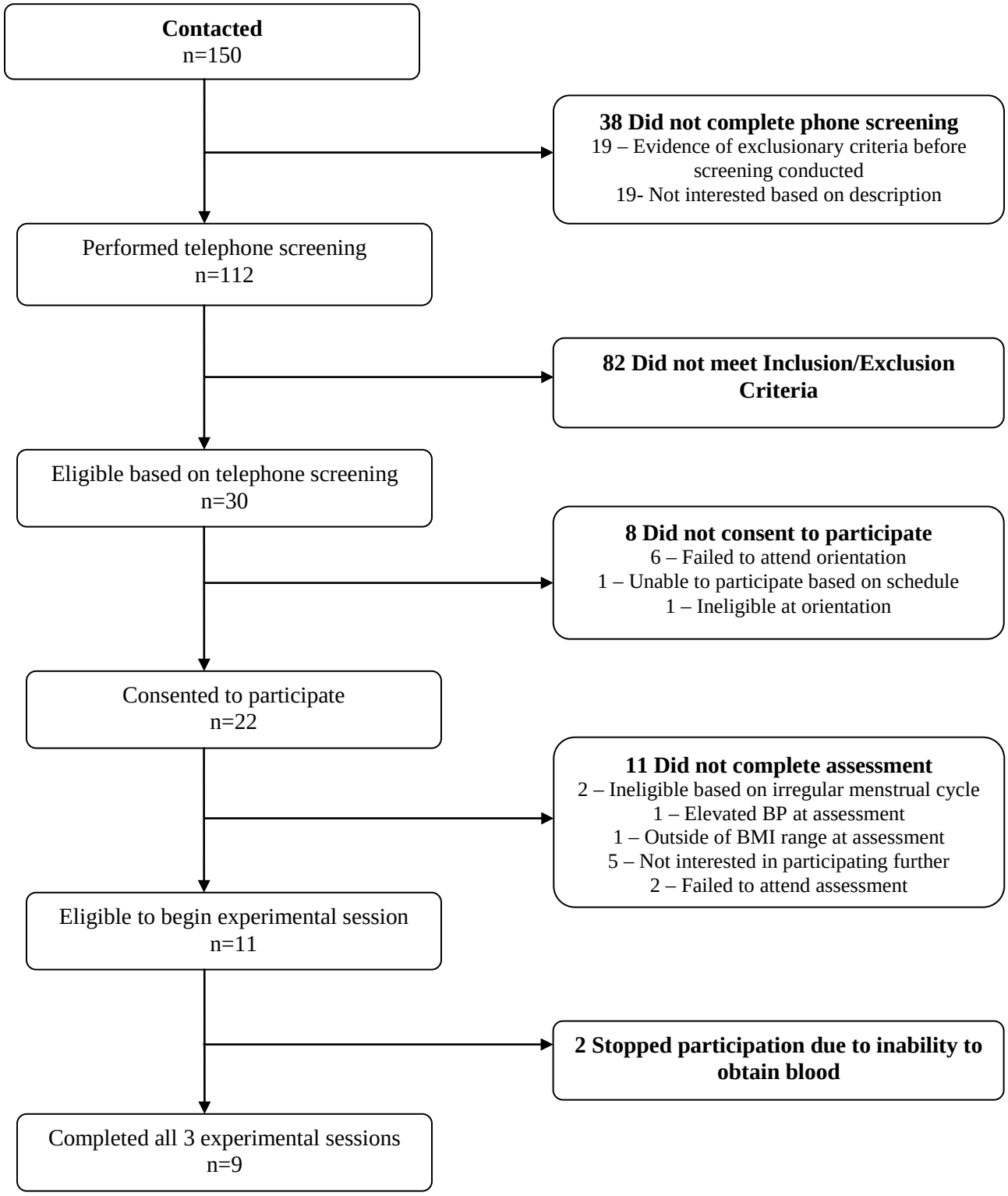


Figure 2 Subject recruitment and enrollment

Table 1 Descriptive variables (mean ± standard deviation)

	All Participants (n=11)	**Completers (n=9)**
Age (years)	46.0 ± 5.4	45.3 ± 5.7
Height (cm)	170.0 ± 9.9	169.7 ± 11.0
Weight (kg)	99.3 ± 13.8	96.7 ± 12.5
BMI (kg/m^2)	34.4 ± 3.7	33.6 ± 3.2
Systolic Blood Pressure (mm/Hg)	122.2 ± 8.6	122.0 ± 8.8
Diastolic Blood Pressure (mm/Hg)	73.9 ± 8.6	73.0 ± 8.9
Waist Circumference (cm)	110.7 ± 11.5	107.7 ± 8.3
VO_2 peak (ml/kg/min)	24.8 ± 6.3	25.4 ± 6.8
Average fasting plasma insulin* (μU/ml)	18.1 ± 7.9	18.1 ± 7.9
Average fasting plasma glucose* (mg/dl)	102.0 ± 13.2	102.0 ± 13.2

*Average from fasting values across all three experimental conditions (REST, 10-EX and 30-EX)

4.2 EXPERIMENTAL SESSIONS

All subjects were able to successfully complete both the 10-minute and 30-minute bouts of treadmill walking during the experimental testing sessions. Heart rate was recorded at the end of each minute during the 10-minute and 30-minute exercise sessions, and then averaged for each exercise session. Subjects were able to walk at a speed and grade that elicited an average of 72±3.5% and 73±1.8% of age-predicted maximal heart rate for the 10-EX and 30-EX sessions,

respectively. A repeated measures ANOVA demonstrated that the average heart rates during the 10-EX and 30-EX exercise sessions were not significantly different (Table 2).

Table 2 Differences in average heart rate across exercise conditions

	10-EX Session (n = 9)	**30-EX Session (n = 9)**	***P*-Value**
Average Heart Rate (bmp)	125 ± 6.0	127 ± 3.5	0.204

(Values presented at mean ± standard deviation)

A repeated measures ANOVA revealed that body weight was not significantly different between the three experimental sessions (Table 3). Additionally, a repeated measures ANOVA demonstrated that fasting insulin and glucose were not statistically different between experimental sessions (Table 3).

Table 3 Differences in fasting weight, insulin and glucose across experimental conditions

	REST Session (n = 9)	**10-EX Session (n = 9)**	**30-EX Session (n = 9)**	***P*-Value**
Fasting Weight (kg)	97.5 ± 12.6	97.3 ± 12.6	97.5 ± 12.0	0.894
Fasting Plasma Insulin (μU/ml)	17.0 ± 7.5	17.5 ± 9.2	20.0 ± 9.5	0.340
Fasting Plasma Glucose (mg/dl)	103.7 ± 14.0	102.6 ± 11.5	99.7 ± 16.0	0.310

(Values presented at mean ± standard deviation)

4.3 COMPARISON OF PLASMA INSULIN AND GLUCOSE ACROSS CONDITIONS

Separate one-way repeated measures ANOVAs were used to assess differences in total plasma insulin AUC and total plasma glucose AUC across the three treatment conditions. Four subjects

were missing a blood sample for at least one time point and were excluded from the AUC analysis. The AUC analysis for plasma insulin and glucose was performed on the 5 subjects with complete data. There was no significant difference in plasma insulin AUC between the REST (12,270±6,148), 10-EX (10,633±5,162), and 30-EX (11,479±4,810) conditions (p=0.354) (Figure 3). The effect size, calculated by Cohen's d, was 0.3 for 10-EX compared to REST, and 0.1 for both REST and 10-EX compared to 30-EX. Similarly, there was no significant difference in plasma glucose AUC between the REST (23,184±6,023), 10-EX (21,735±2,680), and the 30-EX (22,899±3328) conditions (p=0.554) (Figure 4). The effect size for both REST and 30-EX compared to 10-EX was 0.2, and the effect size for REST compared to 30-EX was 0.0.

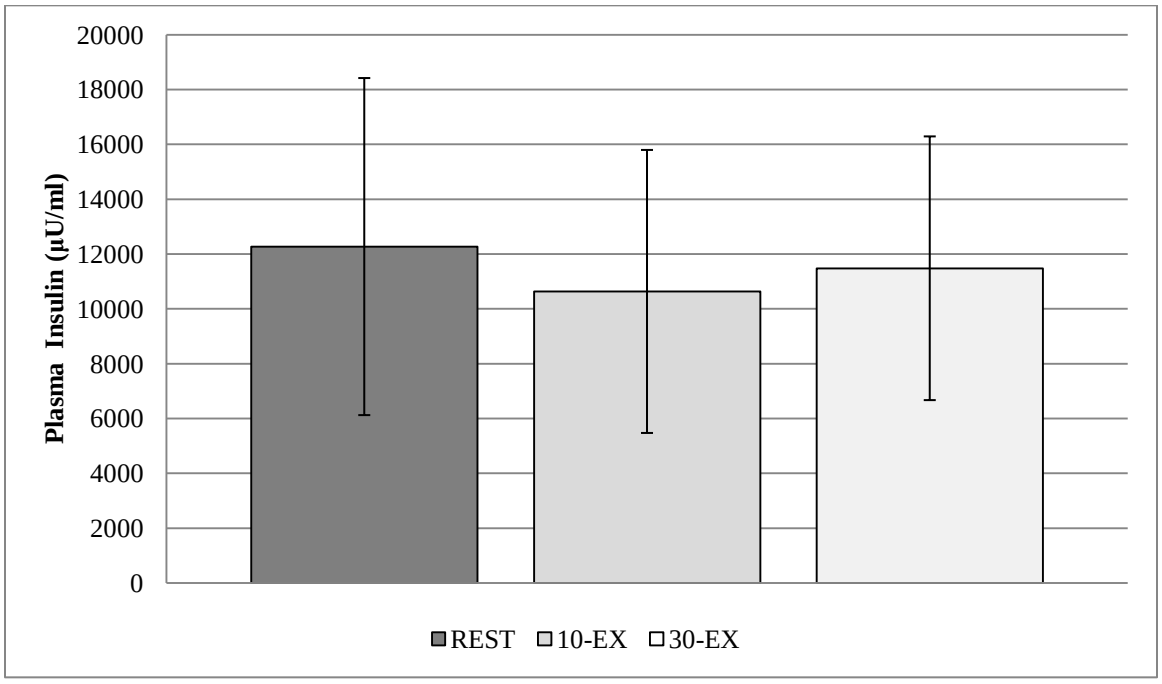


Figure 3 Plasma insulin AUC for each experimental condition (mean ± standard deviation)

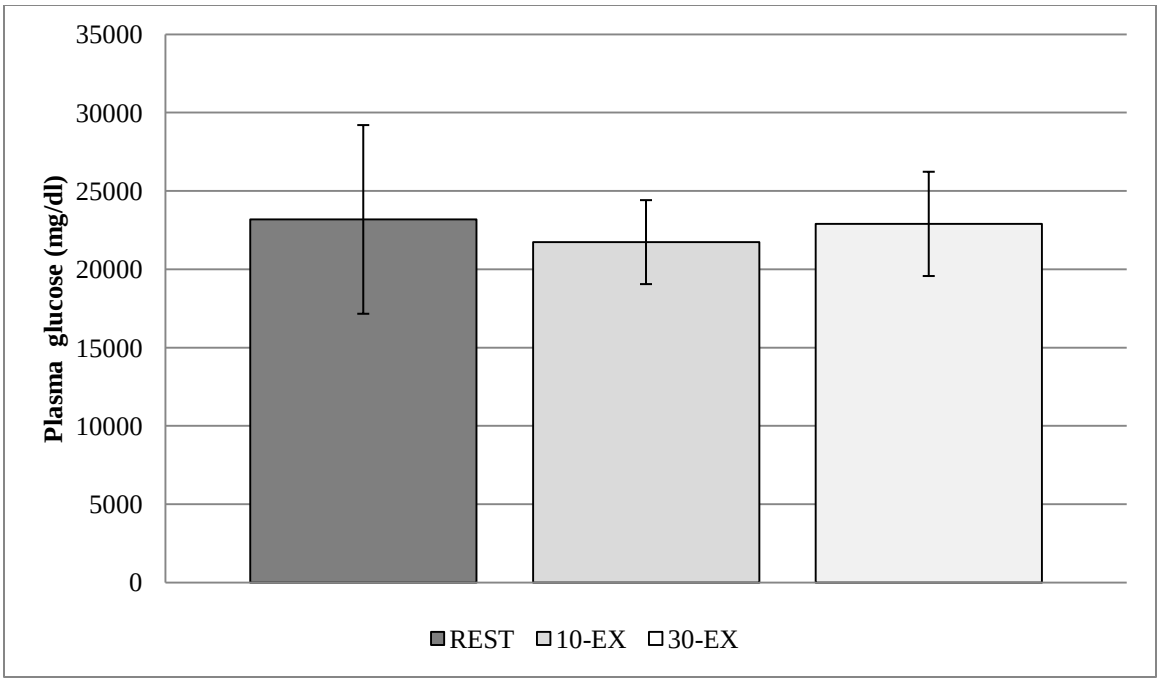


Figure 4 Plasma glucose AUC for each experimental condition (mean ± standard deviation)

To correct for the potential confounding of fasting levels across the three conditions, AUC was also calculated for plasma insulin and glucose by subtracting the AUC below the fasting value (FAST) from the AUC above the fasting value for each condition. For plasma insulin AUC above FAST (Figure 5), there were no significant differences between the three treatment groups (p=0.182). There were also no significant differences between REST, 10-EX or 30-EX for glucose AUC above FAST (p=0.286) (Figure 6).

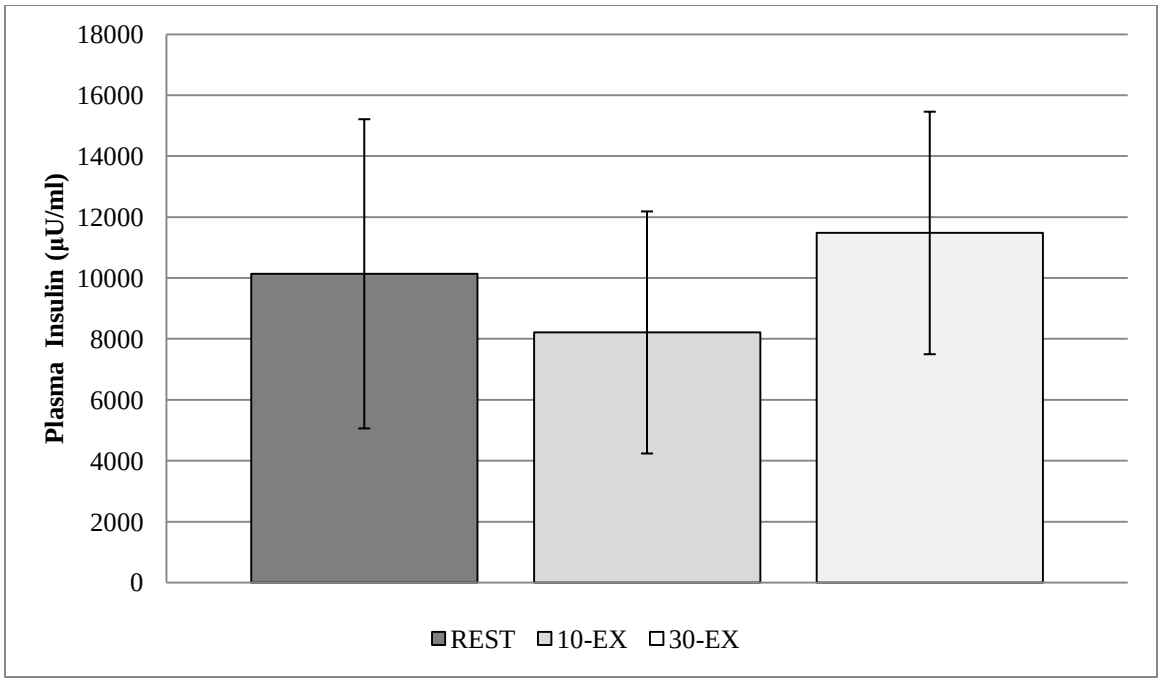


Figure 5 Plasma insulin AUC above FAST (mean ± standard deviation)

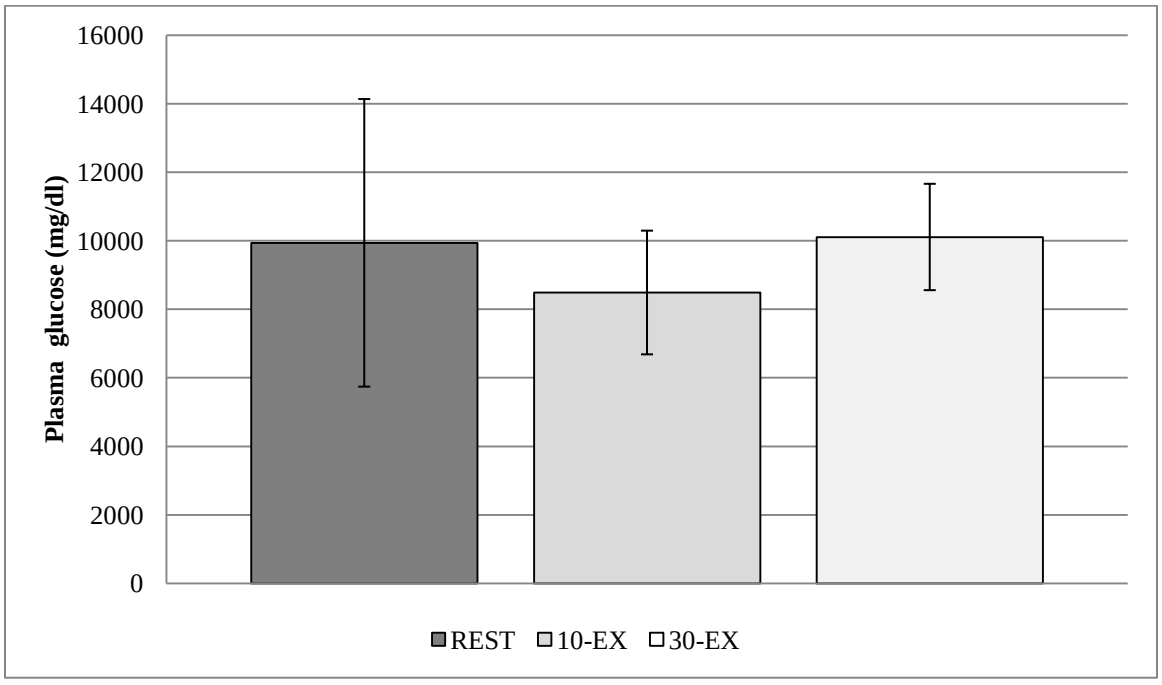
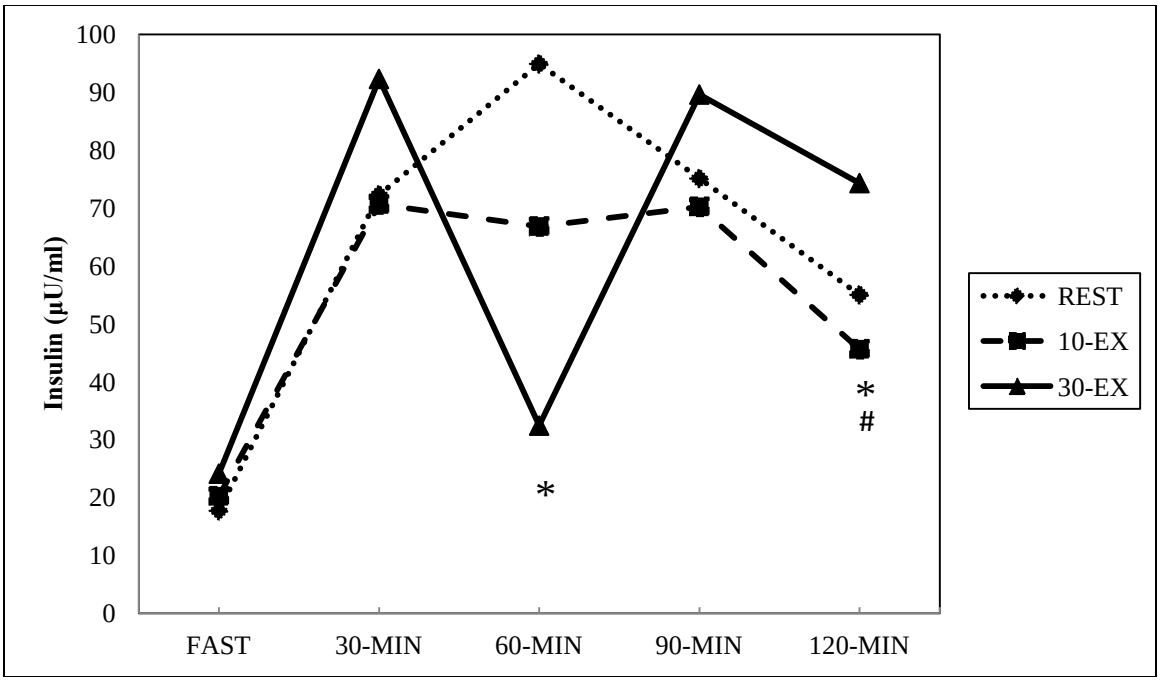


Figure 6 Plasma glucose AUC above FAST (mean ± standard deviation)

While there was no significant difference in total AUC for either insulin or glucose, the patterns of change across time were different among the three experimental conditions (Figures 7

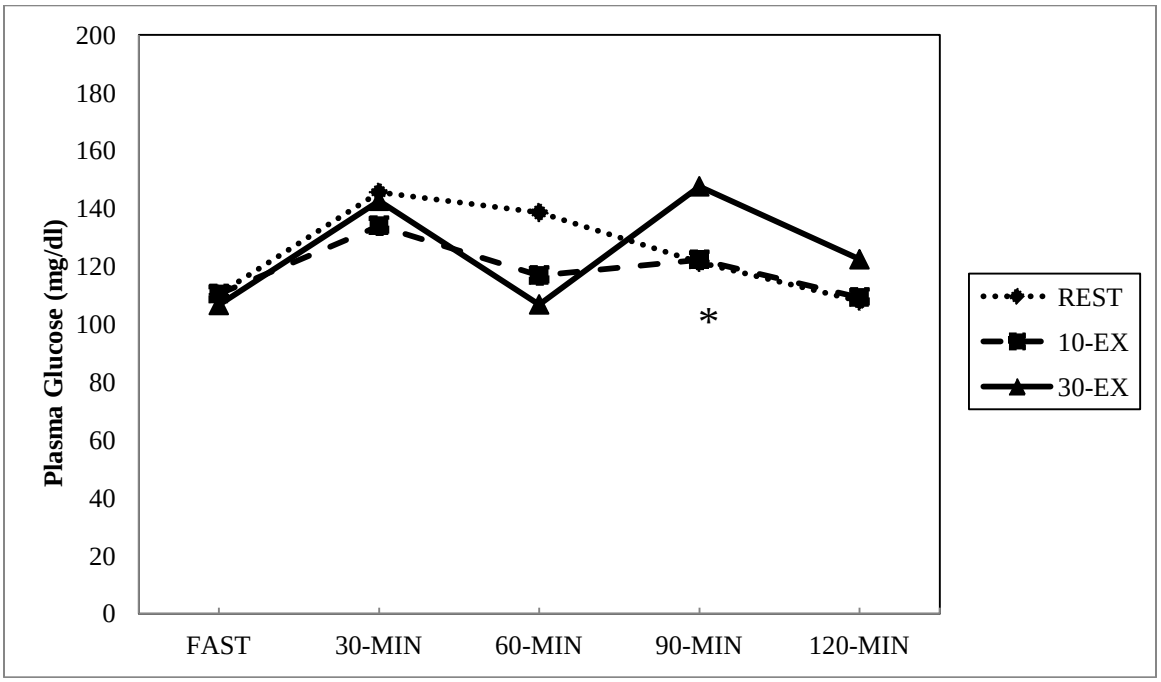
and 8). A one-way repeated measured ANOVA was performed on each time point for plasma insulin and plasma glucose across treatment conditions. Following the breakfast meal, insulin increased similarly from FAST to the 30-minute post breakfast (30-MIN) time point across all three treatment conditions (p=0.72). In the REST condition insulin peaked at 60-minutes post breakfast (60-MIN), but was lower in the 10-EX and 30-EX conditions. A main effect for condition was observed at 60-MIN (p=0.018), but post-hoc analysis using pairwise comparisons with a Bonferroni adjustment did not reveal any significant differences between conditions. From 60-MIN to 90-minutes post breakfast (90-MIN), insulin decreased in REST while a rebound was observed in 30-EX; at 90-MIN there was no significant difference between conditions (p=0.235). Insulin continued to decrease in all groups until 120-minutes post breakfast (120-MIN). Insulin was significantly higher at 120-MIN in 30-EX compared to REST (p=0.042)

A similar pattern was observed for plasma glucose during the three conditions. Glucose increased in all three conditions following breakfast at 30-MIN (p=0.642), and began to decrease in all conditions at 60-MIN (p=0.227). A rebound effect was observed for the 30-EX condition and glucose increased at 90-MIN; however, this pattern was not observed in REST or 10-EX. A significant main effect for condition was observed at 90-MIN (p=0.037), but no differences between groups were detected in post-hoc analyses using pairwise comparison with a Bonferroni adjustment. Glucose decreased in all three groups at 120-MIN similarly in all three conditions (p=0.278)



*Significant main effect for condition
#30-EX significantly different from REST

Figure 7 Pattern of change in plasma insulin across experimental conditions (data is presented for subjects with complete data only)



*Significant main effect for condition

Figure 8 Pattern of change in plasma glucose across experimental condition (data is presented for subject with complete data only)

Table 4 Insulin and glucose at each time point and by experimental condition (data is presented for subjects with complete data only)

		Experimental Condition (n=5)			
Variable	**Time**	**REST**	**10-EX**	**30-EX**	***P*-value**
Plasma Insulin (μU/ml)	FAST	17.7 ± 9.4	20.2 ± 11.0	24.1 ± 10.2	0.279
	30-MIN	72.3 ± 37.3	70.6 ± 32.3	92.3 ± 47.6	0.72
	60-MIN	94.9 ± 48.0	66.8 ± 30.3	32.3 ± 12.3	0.018 [A]
	90-MIN	75.1 ± 41.8	70.2 ± 40.2	89.6 ± 38.3	0.235
	120-MIN	55.4 ± 45.7	45.6 ± 22.7	74.3 ± 45.2[B]	0.045 [A]
Plasma Glucose (mg/dl)	FAST	110.4 ± 15.4	110.4 ± 7.5	106.6 ± 16.4	0.642
	30-MIN	145.6 ± 34.8	134.0 ± 22.9	142.6 ± 26.4	0.138
	60-MIN*	138.6 ± 47.4	116.8 ± 14.6	106.8 ± 8.5	0.102
	90-MIN	121.4 ± 38.0	122.2 ± 21.5	147.6 ± 29.0	0.037 [A]
	120-MIN	108.0 ± 24.0	109.2 ± 11.2	122.4 ± 30.5	0.278

(Data is presented as mean ± standard deviation)
[A] Significant difference among experimental conditions ($p<0.05$)
[B] Significantly different compared to the REST condition
*Variables were not normally distributed. The main effect for condition was determined made using Friedman's test.

4.4 DIFFERENCES IN CHANGE IN INSULIN AND GLUCOSE FROM FASTING

A one-way repeated measures ANOVA was performed for the change in insulin and glucose from fasting at each time point across all three treatment differences (Table 5). All available data was included for each time point. A significant difference between groups was detected for plasma insulin at 60-MIN (p=0.002). Post-hoc analysis revealed that the change in plasma insulin from fasting to 60-MIN was significantly lower in 30-EX (6.1±8.4) compared to both 10-EX (38.3±22.0; p=0.029) and REST (67.4±36.8; p=0.026). There was no significant difference in plasma insulin change between REST and 10-EX for any time points.

Change in plasma glucose from fasting was significantly different across treatment groups at 90-MIN (p=0.003) and 120-MIN (p=0.028) with a trend observed at 60-MIN (p=0.074). Post hoc analysis determined that the change in plasma glucose at 90-MIN from fasting was significantly lower in REST (6.89±22.4; p=0.011) and 10-EX (6.1±18.1; p=0.031) compared to the 30-EX condition (31.6±21.3). At 120-MIN, change in plasma glucose from fasting was also significantly lower in REST (-2.5±20.5; p=0.043) and 10-EX (-1.23±7.1; p=0.043) compared to the 30-EX condition (14.9±17.0). There was no significant difference in plasma glucose change between REST and 10-EX for any time point.

Similar results were observed when analyzing the data by those with complete data only for each time point (Table 4), or when using all available data at each time point expressed as a change from the fasting value (Table 5); discrepancies were observed at only two time points. Specifically, both analyses detected a significant main effect for condition at the 60-MIN time point for plasma insulin and the 90-MIN time point for glucose. However, at the 120-MIN time point for insulin, a significant main effect for treatment was observed only for the analysis on those with complete data. Conversely, a significant main effect for condition was detected at the

120-MIN time point for glucose only in the analysis looking at change from fasting using all available data at that time point. While there are slight differences in the results between analyses, both demonstrate a similar pattern of change in insulin and glucose across experimental sessions. Insulin and glucose appear to decrease during exercise only in 30-EX. After 30 minutes of exercise both insulin and glucose rebound and increase to a value higher than REST or 10-EX, remaining elevated throughout 120-MIN.

Table 5 Change in insulin and glucose from fasting at each time point and by experimental condition

		Experimental Condition (change from fasting)			
Variable	**Time**	**REST**	**10-EX**	**30-EX**	***P*-value**
Plasma Insulin (μU/ml)	FAST	17.0 ± 7.5 (n=9)	17.5 ± 9.2 (n=9)	20.0 ± 9.5 (n=9)	0.340
	Δ30-MIN	61.3 ± 40.6 (n=7)	55.6 ± 30.7 (n=9)	53.0 ± 41.0 (n=8)	0.690
	Δ60-MIN	67.4 ± 36.8 [B] (n=8)	38.3 ± 22.0 [B] (n=9)	6.1 ± 8.4 (n=8)	0.002[A]
	Δ90-MIN	40.7 ± 36.8 (n=9)	39.6 ± 31.0 (n=9)	41.0 ± 42.6 (n=8)	0.910
	Δ120-MIN*	26.8 ± 32.1 (n=8)	22.6 ± 16.0 (n=8)	38.4 ± 36.0 (n=8)	0.115
Plasma Glucose (mg/dl)	FAST	103.7 ± 14.1 (n=9)	102.6 ± 11.5 (n=9)	99.7 ± 16.0 (n=9)	0.310
	Δ30-MIN	31.6 ± 18.6 (n=7)	18.6 ± 20.0 (n=9)	26.5 ± 17.3 (n=8)	0.138
	Δ60-MIN	24.5 ± 28.0 (n=8)	1.2 ± 13.1 (n=9)	0.6 ± 15.6 (n=8)	0.074
	Δ90-MIN	6.89 ± 22.4 [B] (n=9)	6.1 ± 18.1 [B] (n=9)	31.6 ± 21.3 (n=8)	0.003[A]
	Δ120-MIN*	-2.5 ± 20.5 [B] (n=8)	-1.23 ± 7.1 [B] (n=8)	14.9 ± 17.0 (n=8)	0.028 [A]

(Values are presented as mean ± standard deviation)
[A] Significant difference among experimental conditions
[B] Significantly different compared to the 30-EX condition
*Variables were not normally distributed. The main effect for condition was determined made using Friedman's test. Post-hoc comparisons were made using the Wilcoxon signed rank test.

4.5 CORRELATIONS BETWEEN ENERGY EXPENDITURE AND PLASMA INSULIN AND GLCUOSE AUC

While all subjects successfully completed the exercise sessions at an intensity between 70-75% age-predicted maximal heart rate, energy expenditure was not identical between each subjects as each subject walked at a different speed and grade to elicit the target heart rate. To explore whether total energy expenditure was related to differences in plasma insulin or glucose AUC, separate correlations were performed between the energy expenditure for each exercise session and total insulin and glucose AUC (Table 6). Additionally, separate correlations were performed between energy expenditure for each exercise session, and insulin and glucose AUC above FAST (Table 6). 10-EX and 30-EX energy expenditure were not normally distributed, and correlations were calculated using Spearman's rank correlation coefficients (ρ). Correlations were calculated using data from subjects with complete data for the 10-EX (n=8) and 30-EX (n=7) exercise sessions. Energy expenditure was determined using ACSM metabolic equations for estimating energy expenditure.[28]

Significant negative correlations were detected between 10-EX energy expenditure and both total plasma insulin AUC (-0.786; p=0.21) and plasma insulin AUC above FAST (-0.762; p=0.28). Correlations between 10-EX energy expenditure and both glucose AUC and glucose AUC above FAST were not statistically significant. None of the correlations between 30-EX energy expenditure and plasma insulin or glucose AUC were statistically significant.

Additionally, partial correlations were performed adjusting for body weight and fitness, measured as VO_{2peak} in L/min. (Table 6). When adjusted for body weight, energy expenditure remained significantly correlated with plasma insulin AUC in 10-EX. After adjusting for fitness, however, plasma insulin AUC in 10-EX was no longer significantly correlated with energy

expenditure. There were still no significant correlations between 30-EX energy expenditure and plasma insulin or glucose AUC after adjusting for body weight and fitness.

Table 6 Correlations for energy expenditure and insulin and glucose AUC

	10-EX energy expenditure (n=8)			**30-EX energy expenditure (n=7)**		
	Spearman's rank correlation coefficients (ρ)	**Partial Correlation controlling for body weight (kg)**	**Partial Correlation controlling VO2peak (L/min)**	**Spearman's rank correlation coefficients (ρ)**	**Partial Correlation controlling for body weight (kg)**	**Partial Correlation controlling VO2peak (L/min)**
Total Plasma Insulin AUC	-0.786* (p=0.021)	-0.834* (p=0.020)	-0.133 (p=0.809)	-0.000 (p=1.00)	-0.749 (p=0.087)	-0.058 (p=0.913)
Plasma Insulin AUC above FAST	-0.762* (p=0.028)	-0.874* (p=0.010)	-0.048 (p=0.400)	-0.036 (p=0.939)	-0.666 (p=0.149)	-0.167 (p=0.752
Total Plasma Glucose AUC	0.024 (p=0.955)	-0.029 (p=0.951)	0.380 (p=0.809)	0.464 (p=0.294)	0.524 (p=0.286)	0.360 (p=0.483)
Plasma Glucose AUC above FAST	0.048) (p=0.911)	-0.254 (p=0.582)	0.174 (p=0.809)	0.357 (p=0.432)	0.029 (p=0.956)	0.392 (p=0.443)

5.0 DISCUSSION

5.1 SUMMARY OF THE MAIN FINDINGS

The purpose of this study was to compare the effect of 30 minutes of aerobic exercise, 10 minutes of aerobic exercise, and a resting sedentary condition on postprandial plasma insulin and glucose in a group of obese adults. In the present study neither plasma insulin AUC nor plasma glucose AUC was significantly different between any of the experimental conditions. However, the pattern of change for postprandial insulin and glucose was not consistent across these conditions. At 60-MIN, the total change in plasma insulin from fasting was significantly lower in the 30-EX condition compared to the REST and 10-EX condition. At 90-MIN, the REST and 10-EX condition attenuated the increase in glucose seen in the 30-EX condition. Insulin AUC was also significantly correlated total energy expenditure. This relationship remained when adjusting for body weight but not for fitness.

Therefore, the results of the current study indicate that while 30 or 10 minutes of exercise may not significantly reduce plasma insulin or glucose AUC, 30 minutes of exercise appears to affect the pattern of change observed for insulin and glucose differently than either 10 minutes of exercise or a sedentary period. In addition, total change in insulin during and after exercise may also be related to total energy expenditure. It is possible that this relationship is influenced by

fitness, as individuals with greater fitness levels are able to perform more work and expend more energy during a 10-minute exercise session.

5.2 EFFECT OF EXERCISE ON PLASMA INSULIN AND GLUCOSE

The present study is the first to examine the effect of aerobic exercise on postprandial changes in insulin and glucose in a group of obese, nondiabetic adults. Previous research suggests that aerobic exercise significantly reduces postprandial insulin and glucose in diabetic adults.[22, 83-84, 87] For example, Poirier et al. reported that regardless of time since last meal, 60 minutes of aerobic exercise significantly reduced postprandial blood glucose immediately following exercise.[83] Larsen et al. also observed a significant decrease in plasma insulin and glucose AUC following 45 minutes of continuous moderate intensity aerobic exercise.[22] This same group also reported similar changes in insulin and glucose AUC following high intensity exercise consisting of several 4-minute intervals at 100% VO_{2max}.[84] Similarly, van Dijk et al. reported a dose-dependent decrease in postprandial insulin and glucose following 30 and 60 minutes of moderate intensity aerobic exercise.[87] While prior research has observed a decrease in postprandial insulin and glucose following exercise, no study has examined this effect in nondiabetics. Therefore, the purpose of the current study was to determine if similar changes in postprandial insulin and glucose would be observed in a group of obese, nondiabetic adults following acute exercise.

Based on prior research, it was hypothesized that an acute bout of both 10- and 30-minutes of exercise would decrease postprandial insulin and glucose in a dose-dependent manner compared to a resting condition. Results from the current study indicate that plasma insulin and glucose AUC were lower following either a 10- or 30-minute bout of exercise compared to a

resting condition, but this decrease was not statistically significant. Therefore, we failed to reject the null hypothesis that 10 and 30 minutes of exercise would significantly decrease plasma insulin and glucose.

The results from the present study are in contrast to the majority of prior research which observed a significant decrease in postprandial insulin and/or glucose following aerobic exercise. However, the observations in the present study agree with one early study by Caron et al. which examined postprandial changes in insulin and glucose in a group of type 2 diabetics. This group reported a delay in the postprandial insulin response when subjects performed 45 minutes of aerobic exercise at 50% VO_{2max}, but this was delay was not statistically significant. Similarly, a plasma glucose was lower following exercise, but this also failed to reach statistical significance.

5.2.1 Effect of exercise on plasma insulin AUC

The present study did not observe a significantly lower plasma insulin AUC in either exercise condition compared to a resting session in a group of obese, nondiabetic adults. These results are in contrast to previous research reporting decreases in fasting insulin with acute exercise in obese adults.[81, 100] In an early study by Minuk et al., 45 minutes of aerobic exercise at 60% VO_{2max} significantly decreased plasma insulin during exercise.[81] Kang et al. reported similar findings and observed a significant decrease in plasma insulin after both 70 minutes of exercise at 50% VO_{2peak} and 50 minutes of exercise at 70% VO_{2peak}.[100] However, results from the current study are in agreement with a study by Giaccia et al. which also did not observe a significant decrease in insulin following acute exercise.[101] In the trial by Giaccia et al. there was a non-significant trend for lower plasma insulin levels following 45 minutes of aerobic exercise at 50% VO_{2max}. The present study observed similar results (Figure 3), as total plasma insulin AUC was lower in

both 30-EX (11,479±4810) and 10-EX (10,633±5,162) compared to REST (12,270±6,148), but these differences did not reach statistical significance (p=0.354).

The majority of studies examining exercise-induced changes in plasma insulin have been performed in the fasting state, while the current study examined postprandial changes. There is one previous study exploring the effect of acute exercise on postprandial insulin in nondiabetic subjects.[40] In this study by Murphy et al., men and women performed 30 minutes of aerobic exercise at 60% VO_{2max} before breakfast, or 10 minutes of aerobic exercise at the same intensity before breakfast, lunch and dinner. Blood was sampled throughout the day, and there was no difference in plasma insulin concentrations at any time point between the two conditions. Therefore, the current study agrees with the results reported by Murphy et al., suggesting that exercise may not change postprandial insulin in obese adults. However, Murphy et al. included both lean and obese subjects in their sample, making it difficult to compare the findings to the current study. Additionally, the sample size of the current study (n=9; n=5 for complete data at all time points) and the trial by Murphy et al. (n=7) were both small, further limiting interpretation of the results. It is possible that these studies were underpowered to detect a significant difference in insulin between conditions. However, there is also potentially a different mechanism responsible for exercise-induced changes performed in the fasting compared to the postprandial state.

5.2.2 Effect of exercise on AUC for plasma glucose

A decrease in plasma glucose following exercise is well documented in type 2 diabetic subjects.[81-82, 100-101] However, changes in glucose may be less common in obese nondiabetics. Miunk et al. observed decreased glucose in a diabetic subjects but not obese nondiabetics

following 45 minutes of moderate intensity aerobic exercise. [81] Kang et al. also reported a significant decrease in plasma glucose in obese diabetics following exercise at both 50% and 70% of VO_{2peak}, while no change was detected in obese nondiabetics.[100] Similarly, Giacca et al. reported a significant decrease in glucose in obese diabetics but not obese nondiabetics following 45 minutes of aerobic exercise at 50% VO_{2max}.[101] In the current study, plasma glucose AUC was lower in 30-EX (22,899±3,328) and 10-EX (21,735±2,680) compared to REST (23,184± 6,023); however, this difference was not statistically significant (p=0.554).

While the literature suggests that acute exercise may not change fasting plasma glucose in obese adults, no studies have examined this population in the postprandial state. Considering several studies have reported a significant change in postprandial glucose with exercise in diabetics,[22, 83-84] the present investigation examined the effect of exercise on glucose and insulin following a breakfast meal. The exercise sessions in the current study were performed 30 minutes after consuming a meal containing 20% of estimated energy requirement, and a macronutrient distribution of 55% carbohydrate, 30% fat and 15% protein. This energy and macronutrient content is consistent with the meals used in previous studies reporting a significant change in glucose and insulin with exercise.[22, 84]

The disagreement between results in the present study and previous research may be related to differences in fasting glucose levels between subjects. In the current study mean fasting plasma glucose was 102.0±3.2 mg/dl, classifying the population as pre-diabetic. In studies reporting significant changes in postprandial glucose with exercise, mean fasting plasma glucose ranged from 144.4±14.4 mg/dl[87] to 164.0±9.0 mg/dl.[84] It is possible that exercise only decreases blood glucose when basal levels are elevated beyond a certain threshold. Therefore,

the fasting plasma glucose may have been too low in the current sample to observe a significant change due to exercise.

5.2.3 Potential influence of methodological differences between studies

There are methodological differences between the current study and previous research that may provide insight on the differences in findings.

5.2.3.1 Volume of exercise

The current study utilized 10- and 30- minute bouts of acute aerobic exercise at 70-75% age-predicted maximal heart. Thirty minutes was chosen for the longer exercise session as it reflects current guidelines for exercise in healthy adults.[26, 28] These guidelines also suggest that exercise may be accumulated in minimum 10-minute bouts,[26-28] thus 10-minutes was chosen as the duration of the shorter exercise session. While 10- and 30-minute bouts of aerobic exercise agree with current exercise recommendations for health, most prior literature reporting a significant decrease in postprandial insulin and glucose utilized exercise sessions of greater duration and/or intensity than the current study. Poirier et al. observed significant decreases in blood glucose following 60 minutes of exercise at 60% VO_{2peak}.[83] Studies by Larsen et al. reported lower insulin and glucose following 45 minutes of bicycling at 45% $VO_{2max,}$[22] and intermittent high intensity exercise performed as four, 4-minute high intensity intervals at 100% of VO_{2max}.[84] Significant decreases in glucose were also observed by van Dijk et al. following either 30- or 60-minutes of bicycling at 50% of maximal workload.[87] Therefore, 10- or 30-minute bouts of acute moderate intensity exercise may not have been of sufficient duration or total volume to detect a significant decrease in insulin or glucose.

5.2.3.2 Total energy expenditure and muscle glycogen utilization

Insulin secretion is thought to be dependent on total energy availability, indicating that exercise of insufficient volume or total energy expenditure may not decrease insulin and subsequently lower glucose. Larsen et al.[22] have reported results supporting this theory. They observed similar decreases in postprandial insulin following 45 minutes of bicycling at 45% VO_{2max} and after consuming a breakfast that was reduced the number of calories equivalent to expenditure during the exercise session. Results from the current study also observed a relationship between energy expenditure and plasma insulin, with a significant correlation between greater energy expenditure during the 10-EX session and lower insulin AUC observed. Additionally, it is thought that muscle glycogen depletion is related to decreases in insulin. Increases in insulin sensitivity have been correlated to muscle glycogen utilization.[100, 102] Therefore, there may be thresholds for total energy expenditure and glycogen utilization that must occur to significantly reduce plasma insulin or glucose. It is possible that 10 or 30 minutes at 70-75% age-predicted maximal heart rate is below this threshold, which may have influenced plasma insulin or glucose response in this study.

5.2.3.3 Differences in participant characteristics

The present study was the first to examine the effect of acute aerobic exercise on postprandial changes in insulin and glucose in nondiabetic obese subjects. The four studies which previously observed a significant decrease in postprandial insulin and glucose were performed in type 2 diabetics either treated with diet,[22, 84] oral hypoglycemic agents,[83, 87] or insulin therapy.[87] Adults with overt type 2 diabetes are likely more hyperglycemic, hyperinsulinemic and insulin resistant than the pre-diabetic population in the current study. It is possible that hyperglycemia and

hyperinsulinemia must be more severe than what was observed in the population currently studied to produce significant exercise-induced changes in insulin and glucose.

5.2.3.4 Timing of blood collection

The current study collected a blood sample in the fasted state and then fed subjects a breakfast meal. Subjects were observed for 120-minutes following breakfast consumption and blood was collected at 30 minute intervals (Figure 1). The three previous studies utilizing a similar study design sampled blood more frequently than every 30 minutes,[22, 84, 87] and all observed a significant decrease in insulin and glucose when exercise was performed in the postprandial state. Therefore, it is possible that blood was not sampled frequently enough and failed to capture a time point during which significant changes in insulin or glucose were occurring.

5.3 DIFFERENCES IN THE PATTERN OF CHANGE FOR INSULIN AND GLUCOSE

5.3.1 Pattern of change in plasma insulin across experimental conditions

Insulin AUC was not statistically different between REST, 10-EX and 30-EX in the current study. However, the pattern of change between time points was not consistent across the three experiment conditions (Figure 7, Table 4). Insulin increased following the breakfast meal to 30-MIN in all treatment conditions, which was the expected response to the meal. Exercise was initiated at 30-MIN in 10-EX and 30-EX, while subjects remained sedentary in the REST condition. Following exercise, insulin was reduced at 60-MIN in both 10-EX and 30-EX

compared to REST, with a larger decrease observed in 30-EX. There was a significant main effect between conditions (p=0.018) at 60-MIN, but no significant differences were detected with post-hoc comparisons using a Bonferonni adjustment. From 60-MIN to 90-MIN, plasma insulin decreased in REST and stayed relatively stable in 10-EX. In 30-EX, insulin rebounded to a level that was higher, but not statistically different, compared to REST or 10-EX at 90-MIN. Plasma insulin decreased from 90-MIN to 120-MIN in all three groups, and plasma insulin was significantly higher in 30-EX than REST at 120-MIN (p=0.042).

In the current study, this pattern of change for insulin was consistent across the majority of subjects (Appendix N). seven total subjects had complete data for the 30-EX condition, and six of these subjects demonstrated a decrease in insulin during exercise and a subsequent rebound post-exercise. Other studies have reported a similar pattern for insulin following acute aerobic exercise. This "insulin rebound" has been observed under fasting conditions in lean[86] and obese subjects,[81, 86] and during the postprandial period in diabetics.[22, 84]

One potential contributor to this "insulin rebound" is increased sympathetic nervous system activity during exercise. In the study by Larsen et al., plasma catecholamine responses were also measured throughout 45 minutes of exercise and the post-exercise period. They reported that changes in insulin occurred inversely to changes in plasma epinephrine and norepinepherine.[84] The authors concluded that increased sympathetic nervous system activity might depress β-cell secretion of insulin during exercise. Then, catecholamine concentrations decrease at the cessation of exercise and insulin secretion is no longer suppressed, resulting in a rebound in insulin secretion.

While previous research consistently reports a rebound in plasma insulin following aerobic exercise, the exercise sessions utilized in these studies were all a minimum of 30 minutes

in duration.[22, 81, 84, 86] The current study is the first to examine a shorter exercise session, specifically an acute 10-minute bout. In the present study, insulin was reduced during and immediately post-exercise in 10-EX compared to REST, although this difference was not statistically significant. However, the "insulin rebound" observed in 30-EX did not occur in 10-EX. Again, this pattern was consistent across the individual responses for the majority of subjects (Appendix N). Complete data was collected on all but one subject for the 10-EX condition, who was only missing the 120-MIN blood draw. A rebound effect for insulin in 10-EX was only observed in one subject. In the other eight subjects, plasma insulin in 10-EX either continued to decrease or remained stable following exercise. This consistent observation among subjects suggests that the effect of 30-minutes of exercise appears to be different than the effect of 10-minutes on the pattern of change for insulin during and after exercise.

The differences in pattern of change for insulin between a 10- and 30-minute bout of exercise is interesting, particularly the absence of the post-exercise rebound following 10 minutes of exercise. Both exercise sessions were performed at the same intensity; subjects averaged 72±3.5% and 73±1.8% of age-predicted maximal heart rate during exercise in 10-EX and 30-EX, respectively. Therefore, there appears to be a physiological response occurring sometime after 10-minutes of moderate intensity exercise that is responsible for the post-exercise rebound in insulin. Since studies have speculated that catecholamine concentrations might contribute to the insulin rebound,[22, 84] it is possible that 10-minutes of exercise does not elicit a strong enough catecholamine response to result in the "insulin rebound." However, the purpose of this study was not to determine the physiological mechanisms responsible for changes in insulin with exercise. Additional studies are necessary to determine why the "insulin rebound" was not observed following 10 minutes of exercise in the present investigation.

5.3.2 Pattern of change in plasma glucose across experimental conditions

The pattern of change in plasma glucose across the experimental conditions in the current study followed a similar pattern as plasma insulin (Figure 8, Table 4). Glucose increased from FAST to 30-MIN, demonstrating an increase in blood glucose from the meal. Plasma glucose then decreased from 30-MIN to 60-MIN in all conditions. Following exercise, mean plasma glucose was lower in 10-EX and 30-EX compared to REST at 60-MIN, but these differences were not statistically significant. From 60-MIN to 90-MIN, glucose continued to decrease in REST and stayed relatively stable in 10-EX. In 30-EX, plasma glucose rebounded to a much higher level at 90-MIN. This rebound is similar to the pattern observed for plasma insulin in 30-EX at the same time point. A significant main effect for condition (p=0.037) was detected at 90-MIN, with higher mean plasma glucose in 30-EX (147.6±29.0) compared to 10-EX (122.2±21.5) or REST (121.4±38.0). However, post-hoc analysis did not reveal any statistically significant differences between time points. From 90-MIN to 120-MIN glucose decreased in all three conditions. Glucose was higher at 120-MIN in 30-EX compared to 10-EX or REST, although no statistical differences were detected at this time point. This pattern was to similar observations for insulin at the same time points in the present study.

Similar to insulin, the rebound effect observed for glucose in 30-EX was consistent across the majority of subjects in the present study (Appendix O). Complete data was available for 7 subjects for 30-EX, and five of these demonstrated a rebound response for plasma glucose following exercise. Eight subjects had data at all time points for the 10-EX session. In comparison to 30-EX, the pattern for glucose changes was slightly more varied in 10-EX. Upon visual inspection, blood glucose appeared to slightly decrease or stay relatively stable following exercise at 90-MIN in five subjects. A slight rebound effect was observed for plasma glucose in

three of the subjects at 90-MIN, but glucose was never higher in 10-EX compared to 30-EX at any point following exercise in any of the subjects. While statistically significant differences were only detected at 60-MIN for glucose, the consistency in the post-exercise rebound in glucose observed among individual subjects suggests that 30-minutes affects plasma glucose changes differently than 10-minutes of aerobic exercise. Therefore, the individual responses also indicate that a stronger rebound effect is observed following 30 minutes of exercise compared to a 10-minute bout.

5.3.3 Clinical implications of findings

The current study is the first to compare the acute effect a single 10-minute exercise session, a single 30-minute exercise session, and a resting condition on postprandial plasma insulin and glucose in an obese population. Physical activity recommendations suggest aerobic activity can be accumulated in multiple short bouts lasting at least 10 minutes.[26-28] Current research supports the effectiveness of accumulating short bouts of aerobic exercise to improve cardiovascular fitness[29-36] and promote weight loss. [29-31] However, there are few published studies examining the effectiveness of multiple short bouts of exercise on other health outcomes, including insulin and glucose.

The results from the present study suggest that accumulating aerobic activity in multiple 10-minute bouts may be equally or more effective as performing a single 30-minute session to decrease insulin and glucose. The current study observed that insulin and glucose AUC were not statistically different in 10-EX or 30-EX. These results suggest that 30 minutes of exercise is not superior to 10 minutes for decreasing total circulating levels of plasma glucose during and immediately after exercise. However, post-exercise plasma insulin was significantly higher at

120-MIN in 30-EX compared to REST (Figure 7). Therefore, it is possible that performing multiple 10-minute bouts of exercise would prevent the post-exercise rebound in insulin that observed following a single 30-minute bout of exercise.

This study was limited to the effect of a single exercise session. Few studies have reported the chronic effect of performing short bouts of exercise compared to a single, longer bout on changes in insulin and glucose. Donnelly et al. had overweight women accumulate short bouts of activity (two 15-minute sessions per day, five days per week at 50-65% VO_{2max}) or perform a single exercise session (three, 30-minute sessions three times per week at 60-75% VO_{2max}) for 18 months.[32] At the end of the training period, both groups demonstrated lower insulin AUC during an OGTT, but fasting insulin only decreased in the accumulated exercise group. There was no significant decrease in any measures of glucose in either group.

Eriksen et al. performed a similar study in a group of overweight or obese type 2 diabetic males.[37] Subjects completed a single 30-minute exercise session or three, 10-minute exercise sessions per day for 5 weeks. Improvements in fasting glucose and glucose response to an OGTT were only observed in the group performing multiple 10-minute sessions. However, no changes in fasting insulin or insulin response to an OGTT were reported in either group. These two studies indicate that accumulating multiple short bouts of exercise is as effective for managing glycemic control as one longer bout, and may confer some additional benefit.

The current study was not designed to examine the chronic effect of multiple10-minute bouts compared to a single 30-minute bout of exercise on changes in insulin and glucose. However, the present study observed elevated insulin following 30-minutes of exercise, and Donnelly et al. observed a decrease in fasting insulin only in subjects performing multiple short bouts of exercise. [32] Similarly, the results from Erikesen et al. suggest that in diabetics, short

bouts of exercise may more effectively manage hyperglycemia than a single longer bout.[37] It is therefore possible to hypothesize that accumulating short bouts of exercise may be more effective for managing insulin and glucose compared to a single, longer exercise bout.

5.4 LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

This study was designed to examine the effect of 10 and 30 minutes of acute aerobic exercise on plasma insulin and glucose in a group of obese adults. However, there are factors that may have impacted results, and the outcomes should be interpreted accordingly. These limitations are described in the following section. Additionally, recommendations for future research are discussed below:

1. This study was limited to obese (BMI 30.0-<45.0 kg/m^2), sedentary, apparently healthy men and women between the ages of 35 and 55. Therefore, caution should be used when generalizing these findings to other populations, such as normal weight adults or diabetics. Type 2 diabetics are likely to be characterized by more severe hyperglycemia and hyperinsulinemia compared to the population in the current study. It is unknown if 10- and 30-minute bouts would have the same effect on insulin and glucose in diabetics; future studies should examine the effect of 30 minutes versus 10 minutes of exercise on insulin and glucose in subjects with type 2 diabetes.
2. This study was underpowered to detect significant differences in postprandial plasma insulin and glucose between a resting condition, 10-minute exercise condition and a 30-minute exercise condition. It was determined *a priori* that 25 subjects were necessary to detect a significant difference in plasma insulin and glucose. However, 11 subjects were

recruited for this study, 9 completed all three experimental sessions, and blood was obtained at all time points in only 5 subjects. Therefore, the primary analyses were conducted using data from these 5 subjects. Even with the small sample size a significant main effect for condition was detected at 60-MIN and 120-MIN for plasma insulin, and at 90-MIN for glucose (Table 4). In addition, post-hoc analyses reported that plasma insulin was significantly higher in 30-EX compared to REST at 120-MIN, while REST and 10-EX were not significantly different from each other. Additionally, similar patterns in changes for insulin and glucose were observed among the majority of subjects when individual data was reviewed (Appendix N and O). These results suggest that differences may exist in the plasma insulin and glucose response to a 10- and 30-minute bout of exercise. Appropriately powered studies should be conducted in the future to further explore this question.

3. The exercise sessions examined in this study included a 10- and 30-minute bout of treadmill walking at 70-75% age-predicted maximal heart rate. It is possible that this volume of exercise was not enough to elicit a response in plasma insulin and glucose. Other studies reporting significant changes in postprandial plasma insulin and glucose have utilized exercise sessions that were a longer duration and/or a higher intensity than the protocol in the current study.[22, 84, 87] Therefore, future studies should consider using a greater volume of exercise when studying changes in insulin and glucose in obese, nondiabetic adults.
4. Blood draws were obtained using a needle stick at five time points over the course of the testing sessions. For some subjects, multiple attempts were necessary in order to obtain a sample, which delayed the time at which that particular blood draw occurred.

Considering the sensitivity of the time points for changes in insulin and glucose, it is possible that any delays in obtaining blood samples may have impacted the results. Obtaining blood at 60-MIN in the 30-EX condition was particularly difficult since the blood draw occurred immediately upon cessation of exercise. Additionally, blood draws were not completed at all time points for some subjects, resulting in only 5 subjects having complete data. Using an angiocatheter may be a more suitable method for drawing blood for this research design and should be considered in future research.

5. Blood was obtained in the fasting state and then every 30-minutes following a breakfast meal over a 120-minute time period, for a total of five blood draws during each experimental session. Previous research reporting significant changes in postprandial insulin and glucose sampled blood more frequently than every 30 minutes.[22, 84, 87] It is possible that blood should be sampled on a more frequent basis to more accurately determine changes in plasma insulin and glucose. Because this study was unable to use an angiocatheter and required five separate needle sticks it was not feasible to sample at more frequent intervals, primarily due to subject burden. Therefore, future studies may consider using an angiocatheter to obtain blood and draw these samples more frequently than 30-minute intervals in order to increase the likelihood of detecting significant changes in plasma insulin and glucose.
6. This study only explored differences in plasma insulin and glucose with 10-minutes of exercise compared to 30-minutes of exercise. It would be interesting to also assess if there are any differences in affect or subject attitude towards different durations of exercise in future studies. While subjects were not formally surveyed on their feelings or attitudes toward the exercise sessions, several expressed that completing the 30-minute

session was very difficult. These opinions were not expressed as strongly during the 10-minute bout. Although this was an informal observation made by the investigator, it also warrants additional investigation as it may impact the likelihood that individuals would actually perform recommended exercise sessions.

7. This study only examined the acute effect of 10- and 30-minutes of exercise compared to a resting condition. It is unknown how chronic exercise training performed in multiple 10-minute bouts compared to a single 30-minute bout may affect insulin and glucose in obese, non-diabetic adults. Differences in insulin and glucose between these sessions will only contribute to better health outcomes if changes are observed following chronic exercise training. Therefore, future studies should examine the long term effect of performing multiple 10-minute bouts compared to a single 30-minute bout.
8. The current study was not designed to investigate any difference in physiological mechanisms responsible for changes in plasma insulin and glucose between a 10 and 30-minute bout of exercise. This study demonstrated that the pattern of change for both insulin and glucose was different following 10 minutes of exercise compared to 30 minutes of exercise. Therefore, future research should focus on examining the mechanisms responsible for the rebound in insulin and glucose following 30 minutes of exercise that was not observed after the 10 minute session. It is also necessary to determine if this rebound effect following exercise is beneficial or detrimental to any health outcomes, as this would affect how exercise should be prescribed to an obese, non-diabetic population.

5.5 CONCLUSIONS

Obesity is associated with insulin resistance, and accompanying hyperinsulinemia and potentially hyperglycemia. Acute exercise is associated with a decrease in postprandial insulin and glucose in type 2 diabetics, but it is unknown if this same effect occurs in obese, nondiabetic adults. Additionally, physical activity recommendations suggest that aerobic exercise may be accumulated in multiple 10-minute bouts throughout the day. It is currently unknown if 10 minutes of exercise sufficiently decreases insulin and glucose compared to a single 30-minute bout. Therefore, the current study examined the effect of 10 and 30 minutes of acute exercise on postprandial insulin and glucose in obese, nondiabetic adults.

Results from the current study suggest that there is no statistically significant difference in plasma insulin and glucose AUC between 10 and 30 minutes of exercise and a sedentary condition during a 120-minute postprandial period. However, when each time point was analyzed individually, the pattern of change observed for plasma insulin and glucose was not consistent across conditions. Specifically, 30-minutes of exercise decreased plasma insulin and glucose during exercise, with a subsequent increase immediately post-exercise. This rebound in insulin and glucose was not observed in either the 10-minute exercise session or sedentary condition. Therefore, it appears that both 10 and 30 minutes of exercise effectively decrease plasma insulin and glucose. Additionally, it is possible that 10 minutes of exercise may be more beneficial than 30 minutes if there are any negative health outcomes associated with the post-exercise rebound in insulin and glucose. Additional studies are necessary to clarify these findings and determine the chronic effect of performing either a single 30-minute session compared to multiple 10-minute sessions on a daily basis.

In conclusion, while the initial hypothesis that 10- and 30-minutes of aerobic exercise would decrease plasma insulin and glucose AUC was not accepted, additional analyses revealed interesting differences in the pattern of change between the treatment conditions. Specifically, there was a significant main effect for treatment condition at 60-MIN and 120-MIN for insulin, and insulin was significantly greater in 30-EX compared to REST at 120-MIN. For glucose, a significant main effect was detected at 90-MIN. These results suggest that postprandial insulin and glucose decrease to a greater degree during 30-minutes of exercise compared to 10-minutes of exercise or a resting condition, with a subsequent "rebound" observed post-exercise. Additionally, this pattern was consistent among subjects, with insulin and glucose following this pattern in the majority of subjects when observing each subject individually.

Therefore, results from the current study suggest that the differences in pattern of change following 10- and 30-minutes of exercise warrants further investigation. It is possible that this study was underpowered to detect significant differences in plasma insulin and glucose AUC. However, the significant differences in pattern of change between 10- and 30-minutes of exercise observed in this study suggest that the insulin and glucose "rebound" may be the mechanism responsible for lower insulin and glucose following chronic exercise performed in short bouts. As this study was not designed to evaluate the chronic effect of multiple 10-minute versus a single 30-minute bout of exercise, this is an area of research that should be examined in the future as the difference in the pattern of insulin response to 10- and 30-minutes of exercise may have clinical implications for individuals with hyperinsulinemia and insulin resistance.

APPENDIX A

INFORMED CONSENT

University of Pittsburgh

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CONSENT TO ACT AS A SUBJECT IN A RESEARCH STUDY

TITLE: The Effect of 10- versus 30-minutes of Acute Aerobic Exercise on Insulin and Glucose in Obese Adults

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SOURCE OF SUPPORT: Internal Funds, University of Pittsburgh Physical Activity and Weight Management Research Center

DESCRIPTION:

Excess body weight is associated with a number of adverse health consequences including having problems managing your blood sugar levels and increased risk for developing diabetes. Previous research indicates that physical activity can greatly improve the abovementioned conditions. The purpose of this study is to examine the effect of exercise of different durations on your blood sugar levels. Specifically, this study will examine the effect of 10-minutes of exercise compared to 30-minutes of exercise on changes in insulin and glucose in the blood. The results from this study will help better explain whether a short bout of exercise is as effective as a longer bout of exercise for decreasing insulin and glucose in an obese population.

You are being invited to take part in this research because you are within the weight range for the study and do not have any medical conditions that would keep you from participating in moderate intensity exercise in a safe way. Moderate intensity activity is defined as an activity similar to brisk walking during which you can still have a conversation with another person. Men and women invited to participate in the study are between 35-55 years of age. The study will be performed at a University of Pittsburgh facility on a total of 25 individuals.

If you decide to participate in this research study, you will undergo "screening procedures," which are used to determine if you are eligible to participate in this study. It is possible that you may not be eligible to participate in the study after completing one or both of the screening visits. If you are eligible to participate after 2 screening visits, you will be asked to come to the laboratory for 3 experimental sessions. The details of the screening procedures and experimental testing sessions are described below. The following procedures are not part of your standard medical care.

Screening Procedures:

You initially went through a screening procedure over the phone to determine that you are potentially eligible for this study. Two visits to the laboratory are also required in order to perform additional screening procedures to ensure that you are eligible to participate. Specific procedures performed at each screening visit are described below.

Study Orientation/Screening Visit 1

At the first visit, you will be told about the study and presented with this consent form. If you choose to participate in the study, you will then be asked to complete brief screening procedures. First, you will be asked to complete a physical activity readiness questionnaire (PAR-Q), and this will take approximately 5 minutes. You will also complete a detailed medical history, and this will take approximately 20 minutes. These questionnaires will allow the investigators to determine if you have any significant medical condition that would indicate that exercise is unsafe for you. Participants cannot

be pregnant, if you are female you will have a urine pregnancy test prior to starting this study and before each experimental session. The Orientation/Screening visit will take place at the Physical Activity and Weight Management Research Center at the University of Pittsburgh. You will also be required to provide medical clearance from your personal physician before attending the Orientation/Screening visit.

Screening Visit 2

If you remain potentially eligible after the first screening visit, you will be asked to return to the laboratory for a second screening visit to ensure that you are eligible for the study and that it is safe for you to participate in the experimental procedures. Prior to Screening Visit 2, you will be given a list of instructions to follow. These instructions include: fasting for at least 4 hours prior to the visit, avoiding caffeine for 12 hours prior to the visit, avoiding consumption of over-the-counter medications for 24 hours prior to the visit, abstaining from all vigorous physical activity for 24 hours prior to the visit, and transporting yourself to the laboratory in a method that does not allow for excessive physical exertion (i.e., car or bus). Female subjects will have a urine pregnancy test prior to beginning this assessment visit.
The assessment visit will take place at the Physical Activity and Weight Management Research Center at the University of Pittsburgh. The assessments will be completed in approximately 60 minutes (1 hour). A brief description of the assessments follows.

A. Body Weight and Height (5 minutes): Your body weight will be measured using a standard medical scale. Your height will be measured with a ruler that is attached to a flat wall.

B. Blood Pressure and Heart Rate (5 minutes): Your blood pressure and heart rate will be measured using an automatic blood pressure cuff and will follow standard measurement procedures.

C. Waist Circumference (5 minutes): Your waist circumference will be measured using a tape measure.

D. Cardiorespiratory Fitness (30 minutes): Measurement of your cardiorespiratory fitness will provide information about how fit your heart and lungs are to perform exercise. Your fitness will be estimated by having you walk on a treadmill. The speed of the treadmill will be kept at 3.0 mph (a brisk pace). However, the grade of the treadmill will increase 1% every minute so that it feels like you are walking up a hill. As you are walking, your heart rate, blood pressure, and perception of physical exertion will be measured. Your heart rate will be measured using an electrocardiogram, which is also known as an ECG. The ECG will require that electrodes be placed on the chest and abdomen areas of your body. You will continue to walk on this treadmill until you reach a heart rate that is 85 percent of your maximal capacity, which is the highest heart rate you can achieve and is estimated by subtracting your age

from 220 beats per minute, and then the test will be stopped. If you have not reached a heart rate that is 85 percent of your maximal capacity at the highest grade of the treadmill, the speed will gradually increase 0.2 mph each minute until you reach the target heart rate.

During this test, you will breathe in and out through a sterilized (cleaned to prevent the spread of germs and disease) mouthpiece and will wear a set of nose clips so that no air flows through your nose. The air that you breathe will be measured by a machine known as a metabolic cart. This will provide information about the amount of oxygen that you need when you are exercising. A staff member who is certified as an Exercise Specialist by the American College of Sports Medicine and at least one additional staff member will conduct this test. No other study participants will be in the testing room during this assessment. You will be permitted to stop the test at any time if you wish. If during this test it is determined that you have a medical condition that makes it unsafe for you to exercise, you will no longer be permitted to participate in this study, and you will be referred to your primary care physician for medical follow-up.

Experimental Testing Sessions:

If it is found that you are eligible to participate after the two screening visits, you will be asked to schedule three visits to the Physical Activity and Weight Management Research Center. Two visits will require you to exercise and the other will not require you to exercise. These visits will be separated by at least 2 days, and must take place within 31 days of each other. If you are a premenopausal female, these visits must be within days 1-14 of your menstrual cycle. The order in which these visits take place will be randomly determined using a method similar to flipping a coin.

Prior to each experimental session, you will be asked to follow pre-visit instructions which include fasting for 12 hours (avoiding all food and drink other than water), avoiding caffeine for 12 hours, avoiding alcohol for 24 hours, and refraining from exercise for 72 hours prior to the experimental session. You will also be asked to keep a detailed food record for one day prior to the experimental session. Upon arrival at the laboratory, all testing procedures will be reviewed, and each experimental session will take approximately 3 hours to complete. A more detailed description of each of these visits is described below.

1. Resting Condition:
    a. Upon arriving to the laboratory, you will be weighed and all female subjects will have a urine pregnancy test. Blood will then be taken via a needle stick. Approximately 20ml of blood, or about 1.5 tablespoons, will be taken during the blood draw. You will then be fed a breakfast meal, and will be asked to eat the meal within a 15-minute period.

b. Blood will be taken an additional 4 times after you eat the breakfast meal. These will occur at minutes 30, 60, 90 and 120 minutes after eating breakfast. Each blood sample will be approximately 20 ml, or about 1.5 tablespoons. A total of 100 ml, or approximately 8.5 tablespoons will be collected during the entire testing session. Your blood will be analyzed to measure levels of blood glucose and insulin. Blood glucose is the amount of sugar in your blood, and insulin is a hormone that helps regulate blood sugar levels. Blood samples will be obtained by a trained phlebotomist or medical technician.
c. Following the breakfast meal you will then begin a 2-hour resting period. During the rest session you will remain in a seated position and rest quietly for two-hours. You will be provided videos to watch and magazines to read. You may bring your own reading materials, but the investigators must approve them.

B. <u>30-minute Exercise Condition:</u>
   a. Upon arriving to the laboratory, you will be weighed and all female subjects will have a urine pregnancy test. You will also be fitted with a Polar Heart Rate Monitor, which will be worn around your chest, just below your breastbone. Blood will then be taken via a needle stick. Approximately 20ml of blood, or about 1.5 tablespoons, will be taken during the blood draw. You will then be fed a breakfast meal, and will be asked to eat the meal within a 15-minute period.
   b. Blood will be taken an additional 4 times after you eat the breakfast meal. These will occur at minutes 30, 60, 90 and 120 minutes after eating breakfast. Each blood sample will be approximately 20 ml, or about 1.5 tablespoons. A total of 100 ml, or approximately 8.5 tablespoons will be collected during the entire testing session. Your blood will be analyzed to measure levels of blood glucose and insulin. Blood glucose is the amount of sugar in your blood, and insulin is a hormone that helps regulate blood sugar levels. Blood samples will be obtained by a trained phlebotomist or medical technician.
   c. Following the breakfast meal you will rest quietly in a seated position for 30 minutes. During the rest session you will remain in a seated position and rest quietly. You will be provided videos to watch and magazines to read. You may bring your own reading materials, but the investigators must approve them.
   d. You will then walk on the treadmill for 30 minutes. During the 30-minute exercise session you will walk on the treadmill at a speed of 3.0 mph (which is similar to brisk walking) and at an incline that will put your heart rate between 70-75% of you age-predicted maximal heart rate, which is considered to be a moderate intensity. If the incline of the treadmill cannot be adjusted to put your heart rate in the target range, the speed of the treadmill will be reduced until your heart rate is between 70-75% of your age-predicted maximal heart rate. While you are walking, your heart rate

will be monitored continuously using the Polar Heart Rate Monitor. Your airflow will not be obstructed using this procedure.

e. After you have walked on the treadmill for 30 minutes you will rest quietly for a period of 60 minutes. You may watch videos and read provided reading materials or reading materials that have been approved by the investigators.

C. 10-minute Exercise Condition:

a. Upon arriving to the laboratory, you will be weighed and all female subjects will have a urine pregnancy test. You will also be fitted with a Polar Heart Rate Monitor, which will be worn around your chest, just below your breastbone. Blood will then be taken via a needle stick. Approximately 20ml of blood, or about 1.5 tablespoons, will be taken during the blood draw. You will then be fed a breakfast meal, and will be asked to eat the meal within a 15-minute period.

b. Blood will be taken an additional 4 times after you eat the breakfast meal. These will occur at minutes 30, 60, 90 and 120 minutes after eating breakfast. Each blood sample will be approximately 20 ml, or about 1.5 tablespoons. A total of 100 ml, or approximately 8.5 tablespoons will be collected during the entire testing session. Your blood will be analyzed to measure levels of blood glucose and insulin. Blood glucose is the amount of sugar in your blood, and insulin is a hormone that helps regulate blood sugar levels. Blood samples will be obtained by a trained phlebotomist or medical technician.

c. Following the breakfast meal you will rest quietly in a seated position for 30 minutes. During the rest session you will remain in a seated position and rest quietly. You will be provided videos to watch and magazines to read. You may bring your own reading materials, but the investigators must approve them.

d. You will then walk on the treadmill for 10 minutes. During the 10-minute exercise session you will walk on the treadmill at a speed of 3.0 mph (which is similar to brisk walking) and at an incline that will put your heart rate between 70-75% of you age-predicted maximal heart rate, which is considered to be a moderate intensity. If the incline of the treadmill cannot be adjusted to put your heart rate in the target range, the speed of the treadmill will be reduced until your heart rate is between 70-75% of your age-predicted maximal heart rate. While you are walking, your heart rate will be monitored continuously using the Polar Heart Rate Monitor. Your airflow will not be obstructed using this procedure.

e. After you have walked on the treadmill for 10 minutes you will rest quietly for a period of 80 minutes. You may watch videos and read provided reading materials or reading materials that have been approved by the investigators.

RISKS and BENEFITS:

The possible risks of this research study may be due to the exercise that you will be performing and the assessments that will be performed.

Risks:

A. Risks of Exercise: There are rare risks associated with participating in an exercise test. These risks include a serious cardiac (affecting your heart) event, an arrhythmia (your heart beats at a pace that is not normal), or chest pain. An example of a cardiac event would be a heart attack or another medical condition that causes damage to your heart or cardiovascular system. The possibility of experiencing a serious cardiac event has been estimated to be approximately 6 per 10,000 in exercising adults. Therefore, the risk of this happening to you is rare. In addition, during exercise, you may experience an increase in heart rate, an increase in blood pressure, shortness of breath, general fatigue, and in some cases muscle soreness. The risk of this happening to you is likely. Medical equipment will be available during the study sessions in order to monitor heart rate and blood pressure. In the event that you experience a serious medical condition during your exercise session, the session will be stopped and appropriate emergency medical care will be provided. If a serious cardiac event occurs, cardiopulmonary resuscitation (CPR) will be initiated and an automated external defibrillator (AED) will be available for use by certified staff until emergency medical personnel arrive to take over emergency procedures.

B. Risk of Electrocardiogram (ECG): You may experience skin irritation or skin redness from electrodes being placed on your skin. The risk of this happening to you is likely.

C. Risk of having the air that you breathe in and out measured by a metabolic cart: When measuring the air that you breathe in and out during exercise, you may experience a dry mouth, hyperventilation, dizziness, or discomfort. The risk of this happening to you is likely.

D. Risk Associated with wearing the Polar Heart Rate Monitor: Some people may experience discomfort or mild skin irritation where the heart rate monitor is worn. The risk of this happening to you is likely. To help prevent discomfort and mild skin irritation, study staff will use soap and water to clean the elastic strap that attaches the heart rate monitor to your chest and wipe off the heart rate monitor using rubbing alcohol before each use.

E. Risk of Drawing Blood: The risks of drawing blood from a vein include discomfort at the site of puncture and possible bruising and swelling around the puncture site. The risks of this happening to you are likely. Although rare,

it is possible that you may develop an infection or experience faintness from the procedure. The risk of an infection or fainting occurring is rare.

F. Risk of Loss of Confidentiality: There is a possibility of a breach of confidentiality of personal information about you. Procedures that will be completed to protect your confidentiality are listed below.

Benefits:

There are no direct benefits that you will receive from participating in this study. If we should find out about a medical condition you were unaware of, with your written permission, this information will be shared with the doctor of your choice. There are general benefits associated with your participation, such that the results from this study will allow investigators to make better recommendations for exercise for obese individuals.

NEW INFORMATION:

You will be promptly notified if any new information develops during the conduct of this research study, which may cause you to change your mind about continuing to participate.

COSTS and PAYMENTS:

Neither you, nor your insurance provider, will be charged for the costs of any of the procedures performed for the purpose of this research study. These costs will be paid by the sponsor of this research study.

You will be paid $300 upon completion of all testing procedures which include the study orientation/screening visit 1, screening visit 2, and the 3 experimental testing sessions described above. Thus, a total of $300 can be earned for your participation in this study. There is no payment for only completing a portion of the study. You will be provided free parking for all study visits.

COMPENSATION FOR INJURY:

If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. It is possible that UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the

costs of this follow-up care unless otherwise specifically stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

CONFIDENTIALITY:

Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. In addition, all research databases will have password controlled access, and this will be controlled by the researchers. Only the researchers listed on the first page of this form and their staff will have access to your research records. However, other scientists may request data obtained by this study. We will allow data to be released to qualified researchers only after ensuring that your name and other identifying information is not given to these researchers. You will not be identified by name in any publication of research results unless you sign a separate form giving your permission (release).

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, your research records may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the researchers learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

A cardiologist in the University of Pittsburgh School of Medicine and UPMC will review the exercise tests that are completed as part of your participation in this study, and he/she will have access to your identifiable medical information.

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for 7 years following the completion of this study, as per University policy.

University Of Pittsburgh Institutional Review Board
Approval Date: 4/15/2012 Renewal Date: 1/16/2013
IRB #: PRO11120303

RIGHT TO PARTICIPATE or WITHDRAW FROM PARTICIPATION:

Your participation in this research study is completely voluntary. Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

You may withdraw, at any time, your consent for participation in this research study. Any identifiable research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

It is possible that you may be removed from the research study by the researchers if, for example, your health status changes and it does not appear that it is safe for you to continue to exercise. You will also be removed if you should become pregnant during this study.

****

VOLUNTARY CONSENT

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed a listed investigator. I understand that I may contact the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

Participant's Signature	Date

CERTIFICATION OF INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual, and I have discussed the potential benefits and possible risks of study participation. Any questions the individual has about this study have been answered, and we will always be available to address future questions, concerns or complaints as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

Printed Name of Person Obtaining Consent	Role in Research Study
Signature of Person Obtaining Consent	Date

APPENDIX B

PHYSICIAN CONSENT

PHYSICIAN CONSENT TO PARTICIPATE IN A DIET AND EXERCISE PROGRAM AT THE UNIVERSITY OF PITTSBURGH

TO: _____

Physician's Name

Address

City State Zip

( )

Telephone Number

RETURN TO: (envelope provided)
Anne Mishler, M.S.
University of Pittsburgh
Department of Health and Physical Activity
Physical Activity and Weight Management Research Center
2100 Wharton Street, Suite 600
Pittsburgh, PA 15203
Telephone: (412) 488-4184
FAX: (412) 488-4174

Your patient _____ has asked to participate in an exercise study at the University of Pittsburgh. This is study is examining the effect of different durations of exercise (10 minutes compared to 30 minutes). Subjects cannot be diagnosed with Type I or Type II diabetes. This study will involve the following.

1. A graded exercise test which involves walking on a motorized treadmill, with the workload gradually increasing every minute. The test will be terminated when the patient achieves 85% of their age-predicted maximal heart rate, or prior to this level if the individual experiences signs or symptoms that would indicate that exercise is contraindicated. Both blood pressure and heart rate will be monitored continuously. The ACSM Guidelines for Exercise Testing will be followed.
2. Two exercise testing conditions at 70% to 75% of age-predicted maximal heart rate. One exercise session will be 10 minutes in duration, and the other will be 30 minutes in duration.
3. A list of additional factors that are exclusionary criteria for this study that you should consider are listed on the attached sheet.

****

Please indicate below if this program seems appropriate for your patient or if you see any contraindications for her participation (*please check the appropriate box below)*.

☐ I verify that this patient has not been diagnosed with Type I or Type II Diabetes and know of no contraindications to this patient participating in any of the above components of the program.

☐ I feel that this program would not be appropriate for this patient for the following reason(s):

_____

_____ _____

Signature of Physician Date

Please consider the following Inclusion and Exclusion Criteria as you evaluate whether your patient is capable of safely participating in the weight loss and exercise research study at the University of Pittsburgh.

Inclusion Criteria:	**Exclusion Criteria:**
• Female or Male • 35-55 years of age • BMI = 30.0-<45.0kg/m^2 • Sedentary for the past 6 months (performing less than 60 minutes of exercise per week over the past 6 months) • Ability to provide consent from their personal physician to participate in this study. •	• Previous diagnosis of cancer, heart disease, Type I or Type II diabetes, or polycystic ovarian syndrome. • Taking prescription or over-the-counter medications that affects glucose metabolism, insulin, blood pressure or heart rate. • Presence of any condition that may limit one's ability to walk for exercise (e.g., orthopedic limitations or severe arthritis). • Currently participating in a weight loss program or reporting significant weight loss (>3.0% of body weight) in the past month. • Women who are currently pregnant (all females will have a urine pregnancy test prior to participating in all experimental sessions) • Having a resting systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg or currently taking prescription medication to control blood pressure. • Premenopausal women reporting irregular menstrual cycles (<25 days or >35 days between cycles). • Report consuming more than a moderate amount of alcohol (>7 drinks per week for females and >14 drinks per week for males)

APPENDIX C

ASSESSMENT INSTRUCTIONS

Dear Participant,

Thank you again for participating in the Exercise Study. At this point, you have completed the orientation session and initial screening questions. You will now need to come back to the laboratory for an assessment visit. Your assessment visit is scheduled for:

_____

If at any point you realize that you need to reschedule, please call our office immediately at 412-488-4170. Assessment times are limited at our center, thus, it is extremely important that you make a strong effort to attend when scheduled.
If at any point you realize that you need to reschedule, please call our office immediately at 412-488-4170.

Prior to your visit, we ask that you adhere to the following guidelines:

1. Abstain from caffeine for at least 4 hours.
2. Fast for at least 4 hours (no food or drink other than water).
3. Avoid consumption of over-the-counter medications for 24 hours.
4. Refrain for vigorous physical activity for 24 hours.
5. Transport yourself to the Physical Activity and Weight Management Research Center in a method that does not allow for excessive physical exertion (i.e., car or bus).

Be sure to report to our center ***with clothing and shoes that are comfortable for exercising***. Please make sure you wear closed toed shoes. You may wear these clothes to the center, or you can use our changing facilities when you get here. If you have any questions about any of these guidelines, please feel free to contact Annie Mishler at 412-488-4170. We look forward to seeing you at your scheduled visits!

APPENDIX D

ASSESSMENT COMPLIANCE QUESTIONNAIRE

Exercise Study
Assessment Compliance Questionnaire

Subject ID: _____ Date _____

Please ask the subject the following questions:

- Have you fasted for at least 4 hours? Yes No
- Have you avoided consumption of over-the-counter medications for 24 hours prior to the visit? Yes No
- Have you abstained from all vigorous physical activity for 24 hours prior to the visit? Yes No
- Did you transport yourself to the lab in a vehicle? Yes No

APPENDIX E

ASSESSMENT DATA COLLECTION FORM

"Exercise and Insulin"
Assessment Form

Subject Number: _____ Acrostic: _____

The assessments must be completed in the following order by the individuals listed. *Under no circumstances is this protocol to be altered unless approved by John/Annie for this participant*.

****Mark each item as it is completed.*

- ☐ Greet participant Initial: _____
- ☐ Blood Pressure/Heart Rate Initial: _____
- ☐ Height, Weight, Waist Circumference Initial: _____
- ☐ Exercise test prep Initial: _____
- ☐ Exercise test Initial: _____

"Exercise and Insulin"
Assessment Form

Subject Number: _____ **Date:** ___/___/___

Acrostic: _____

Assessment Period: ___**Baseline**

Height: _____ **cm (measure to the nearest 0.1 cm)**

Weight: _____ **kg (read from digital scale to the nearest 0.1 kg)**

BMI: _____ **kg/m^2**

Blood Pressure and Heart Rate

Are you taking blood pressure medication? ___**Yes** ___**No**

Time: _____ ___**am** ___**pm**

Arm Circumference: _____**cm**

Cuff Size:

___**Regular** **(24.0 - 32.9 cm)**
___**Large** **(33.0 - 41.0 cm)**
___**Thigh** **(> 41.0 cm)**

	*Systolic Pressure	**Diastolic Pressure
First Reading		
Second Reading		
***Third Reading**		

***The two Systolic Pressures should differ by ≥ 10 mmHg**
****The two Diastolic Pressures should differ by ≥ 6 mmHg**
***** If above criteria are not met take additional readings until these criteria are met.**

	Heart rate (read from Dinamap)
First Reading	
Second Reading	
***Third Reading**	

"Exercise and Insulin"
Assessment Form

Subject Number: _____ **Date:** ___/___/___

Acrostic: _____

Assessment Period: ___**Baseline**

Additional Information for GXT:

Age:_____ years

Date of birth: ___/___/_____ (month/day/year)

Girths
(Measured in centimeters to 1 decimal point)

	1st Measurement	2nd Measurement	*3rd Measurement
Waist *(taken at the level of the iliac crest)*			

***Take a third measurement only if the difference between the first and second measurement is > 1.0cm.**

Urine Pregnancy Test Completed (females only): ____ **Yes** ____ **No**

If "No" explain why: _____

Results of Urine Pregnancy Test: ____**Positive (do not proceed)**
____**Negative**

Graded Exercise Test

ID#:_____ Date: _____

Age: _____ years Height: _____ cm Weight: _____ lbs

Termination Heart Rate: _____ bpm (85% of Age-Predicted Max Heart Rate)

Time (minutes)	Speed (mph)	%Grade	Heart Rate (bpm)	Blood Pressure	RPE
0:00-1:00	3.0	0.0%		XXXXX	XXXXX
1:01-2:00	3.0	1.0%		/	
2:01-3:00	3.0	2.0%		XXXXX	XXXXX
3:01-4:00	3.0	3.0%		/	
4:01-5:00	3.0	4.0%		XXXXX	XXXXX
5:01-6:00	3.0	5.0%		/	
6:01-7:00	3.0	6.0%		XXXXX	XXXXX
7:01-8:00	3.0	7.0%		/	
8:01-9:00	3.0	8.0%		XXXXX	XXXXX
9:01-10:00	3.0	9.0%		/	
10:00-11:00	3.0	10.0%		XXXXX	XXXXX
11:01-12:00	3.0	11.0%		/	
12:01-13:00	3.0	12.0%		XXXXX	XXXXX
13:01-14:00	3.0	13.0%		/	
14:01-15:00	3.0	14.0%		XXXXX	XXXXX
15:01-16:00	3.0	15.0%		/	
16:01-17:00	3.0	16.0%		XXXXX	XXXXX
17:01-18:00	3.0	17.0%		/	
18:01-19:00	3.0	18.0%		XXXXX	XXXXX
19:01-20:00	3.0	19.0%		/	
20:01-21:00	3.0	20.0%		XXXXX	XXXXX
21:01-22:00	3.0	21.0%		/	
22:01-23:00	3.0	22.0%		XXXXX	XXXXX
23:01-24:00	3.2	22.0%		/	
24:01-25:00	3.4	22.0%		XXXXX	XXXXX
25:01-26:00	3.6	22.0%		/	
26:01-27:00	3.8	22.0%		XXXXX	XXXXX
27:01-28:00	4.0	22.0%		/	
28:01-29:00	4.2	22.0%		XXXXX	XXXXX
29:01-30:00	4.4	22.0%		/	
Termination Time: ___:___				/	

Recovery:

0:00-1:00	2.5	0.0%		XXXXX	XXXXX
1:01-2:00	2.0	0.0%		/	XXXXX
2:01-3:00	1.5	0.0%		XXXXX	XXXXX
3:01-4:00	Seated	Seated		/	XXXXX
4:01-5:00	Seated	Seated		XXXXX	XXXXX
5:01-6:00	Seated	Seated		/	XXXXX
6:01-7:00	Seated	Seated		XXXXX	XXXXX

Reasons the Test was Terminated:

APPENDIX F

TESTING SESSION INSTRUCTIONS

Dear Participant,

Thank you again for participating in the Exercise Study. At this point, you have completed the initial assessment visits and now have three, 3-hour testing sessions that must be performed prior to completion of this study. Your 3 testing sessions have been scheduled for:

1. _____
2. _____
3. _____

If at any point you realize that you need to reschedule, please call our office immediately at 412-488-4170.

Prior to your visit, we ask that you adhere to the following guidelines:

6. Refrain from exercise for 72 hours prior to the experimental session.
7. Keep a detailed food record for one day prior to the experimental session.
8. Avoid alcohol for 24 hours prior to the experimental session.
9. Abstain from all food and drink other than water for 12 hours prior to the experimental session. This includes abstaining from all caffeinated beverages as well as chewing gum and mints.

Be sure to report to our center ***with clothing and shoes that are comfortable for exercising***. Please make sure you wear closed toed shoes. You may wear these clothes to the center, or you can use our changing facilities when you get here. If you have any questions about any of these guidelines, please feel free to contact Annie Mishler at 412-488-4170. We look forward to seeing you at your scheduled visits!

APPENDIX G

EXPERIMENTAL SESSION COMPLIANCE QUESTIONNAIRE

Experimental Session Compliance Questionnaire

Subject ID:_____ Date:_____

Session (*circle*): REST 10-EX 30-EX

Please ask the subject the following questions:

Have you exercised in the past 2 days?	□YES	□NO
Have you consumed anything other than water in the past 12 hours?	□YES	□NO
Did you consume alcohol in the past 24 hours?	□YES	□NO
Did you transport yourself to the lab in a vehicle?	□YES	□NO
Did you keep your food record yesterday?	□YES	□NO

APPENDIX H

RESTING SESSION DATA COLLECTION FORM

IRB #: PRO11120303 The Effect of 10- versus 30-minutes of Acute Aerobic Exercise on Insulin and Glucose in Obese Adults
PI: Anne Mishler, MS

Subject ID_____ **Experimental Session Number** (*circle*): 1 2 3

Resting Session (REST)

Date_____

Study Procedures	**Notes**	**Criteria Met Y/N**
Confirm pre-visit compliance to directives		
Weigh Subject	Today's Weight _____	
Direct subject to blood draw/resting area		
Perform fasting blood draw		
Feed subject breakfast (maximum time allowed to eat is 15 mintues):	If experimental session 1, record time to eat breakfast: _____ If experimental session 2 or 3, record time allowed to eat breakfast (from experimental session 1: _____) If experimental session 2 or 3, record time the subject took to eat breakfast: _____	
Record Time of day that subject finishes breakfast (***0-minute time point***)	Time: _____	
Perform 30-minute post-breakfast blood draw and record time	Time:_____	
Perform 60-minute post-breakfast blood draw and record time	Time:_____	
Perform 90-minute post-breakfast blood draw and record time	Time:_____	

IRB #: PRO11120303 The Effect of 10- versus 30-minutes of Acute Aerobic Exercise on Insulin and Glucose in Obese Adults
PI: Anne Mishler, MS

Subject ID_____ **Experimental Session Number** (*circle*): 1 2 3

Perform 120-minute post-breakfast blood draw and record time	Time:_____	
Remove heart rate monitor		

If experimental session 1 or 2:

Schedule next session (if necessary) or confirm date of next scheduled session		
Review and distribute: pre-visit instructions and food diary		

If experimental session 3:

Distribute and explain We-Pay card		

Form Completed by: _____Date_____

APPENDIX I

10-EX SESSION DATA COLLECTION FORM

IRB #: PRO11120303 The Effect of 10- versus 30-minutes of Acute Aerobic Exercise on Insulin and Glucose in Obese Adults
PI: Anne Mishler, MS

Subject ID_____ **Experimental Session Number** (*circle*): 1 2 3

10-minute Exercise Session (10-EX)

Date_____

Study Procedures	**Notes**	**Criteria Met Y/N**
Confirm pre-visit compliance to directives		
Weigh Subject	Today's Weight _____	
Attach heart rate monitor		
Direct subject to blood draw/resting area		
Perform fasting blood draw		
Feed subject breakfast (maximum time allowed to eat is 15 mintues):	If experimental session 1, record time to eat breakfast: _____ If experimental session 2 or 3, record time allowed to eat breakfast (from experimental session 1: _____) If experimental session 2 or 3, record time the subject took to eat breakfast: _____	
Record Time of day that subject finishes breakfast (***0-minute time point***)	Time: _____	
Perform 30-minute post-breakfast blood draw and record time	Time:_____	
Direct subject to treadmill room		

IRB #: PRO11120303 The Effect of 10- versus 30-minutes of Acute Aerobic Exercise on Insulin and Glucose in Obese Adults
PI: Anne Mishler, MS

Subject ID_____ **Experimental Session Number** (*circle*): 1 2 3

Begin 10-minute walking period at 3.0 mph and a grade that induces a heart rate between 70-75% of age-predicted max		
Monitor and record heart rate every minute on testing form and adjust grade/speed as necessary		
Direct subject back to blood draw/resting area		
Perform 60-minute post-breakfast blood draw and record time	Time:_____	
Perform 90-minute post-breakfast blood draw and record time	Time:_____	
Perform 120-minute post-breakfast blood draw and record time	Time:_____	
Remove heart rate monitor		

If experimental session 1 or 2:

Schedule next session (if necessary) or confirm date of next scheduled session		
Review and distribute: pre-visit instructions and food diary		

If experimental session 3:

Distribute and explain We-Pay card		

APPENDIX J

10-EX SESSION TREADMILL DATA COLLECTION FORM

10-minute Exercise Session (EX-10) Data Sheet

Subject ID: _____ Visit #: _____ Date: _____

Predicted HR_{max}: _____ Target HR range (70-75% max HR): _____

Starting TM grade: _____ **Starting TM speed: 3.0 mph**

Time	% Grade	HR	*Grade Adjusted (Y/N)*
BL (30 min Rest)			
0 -1 min			
1 - 2 min			
2 - 3 min			
3 - 4 min			
4 - 5 min			
5 - 6 min			
6 - 7 min			
7 - 8 min			
8 - 9 min			
9 - 10 min			

The treadmill grade/speed will be adjusted using the following protocol:

1) If the heart rate is below the range of 70% to 75% of age-predicted maximal heart rate for two consecutive minutes increase the grade by 1.0%

2) If the heart rate is above the range of 70% to 75% of age-predicted maximal heart rate for two consecutive minutes decrease the grade by 1%.

3) If a grade of 0% elicits a heart rate above the range of 70% to 75% of age-predicted maximal heart rate for two consecutive minutes decrease the speed by 0.2 mph.

APPENDIX K

30-EX SESSION DATA COLLECTION FORM

IRB #: PRO11120303 The Effect of 10- versus 30-minutes of Acute Aerobic Exercise on Insulin and Glucose in Obese Adults
PI: Anne Mishler, MS

Subject ID_____ **Experimental Session Number** (*circle*): 1 2 3

30-minute Exercise Session (30-EX)

Date_____

Study Procedures	**Notes**	**Criteria Met Y/N**
Confirm pre-visit compliance to directives		
Weigh Subject	Today's Weight _____	
Attach heart rate monitor		
Direct subject to blood draw/resting area		
Perform fasting blood draw		
Feed subject breakfast (maximum time allowed to eat is 15 mintues):	If experimental session 1, record time to eat breakfast: _____ If experimental session 2 or 3, record time allowed to eat breakfast (from experimental session 1: _____) If experimental session 2 or 3, record time the subject took to eat breakfast: _____	
Record Time of day that subject finishes breakfast (***0-minute time point***)	Time: _____	
Perform 30-minute post-breakfast blood draw and record time	Time:_____	
Direct subject to treadmill room		

Subject ID_____ **Experimental Session Number** (*circle*): 1 2 3

Begin 30-minute walking period at 3.0 mph and a grade that induces a heart rate between 70-75% of age-predicted max		
Monitor and record heart rate every minute on testing form and adjust grade/speed as necessary		
Direct subject back to blood draw/resting area		
Perform 60-minute post-breakfast blood draw and record time	Time:_____	
Perform 90-minute post-breakfast blood draw and record time	Time:_____	
Perform 120-minute post-breakfast blood draw and record time	Time:_____	
Remove heart rate monitor		

If experimental session 1 or 2:

Schedule next session (if necessary) or confirm date of next scheduled session		
Review and distribute: pre-visit instructions and food diary		

If experimental session 3:

Distribute and explain We-Pay card		

APPENDIX L

30-EX SESSION TREADMILL DATA COLLECTION FORM

30-minute Exercise Session (EX-30) Data Sheet

Subject ID: _____ Visit #: _____ Date: _____

Predicted HR_{max}: _____ Target HR range (70-75% max HR): _____

Starting TM grade: _____ **Starting TM speed: 3.0 mph**

Time	% Grade	HR	*Grade Adjusted (Y/N)*
BL (30 min Rest)			
0 -1 min			
1 - 2 min			
2 - 3 min			
3 - 4 min			
4 - 5 min			
5 - 6 min			
6 - 7 min			
7 - 8 min			
8 - 9 min			
9 - 10 min			
10 - 11 min			
11 - 12 min			
12 - 13 min			
13 - 14 min			
14 - 15 min			
15 - 16 min			
16 - 17 min			
17 - 18 min			
18 - 19 min			
19 - 20 min			
20 - 21 min			
21 - 22 min			
22 - 23 min			
23 - 24 min			
24 - 25 min			
25 - 26 min			
26 - 27 min			
27 - 28 min			
28 - 29 min			
29 - 30 min			

The treadmill grade/speed will be adjusted using the following protocol:

1) If the heart rate is below the range of 70% to 75% of age-predicted maximal heart rate for two consecutive minutes increase the grade by 1.0%

2) If the heart rate is above the range of 70% to 75% of age-predicted maximal heart rate for two consecutive minutes decrease the grade by 1%.

3) If a grade of 0% elicits a heart rate above the range of 70% to 75% of age-predicted maximal heart rate for two consecutive minutes decrease the speed by 0.2 mph.

APPENDIX M

MENU FOR BREAKFAST MEAL

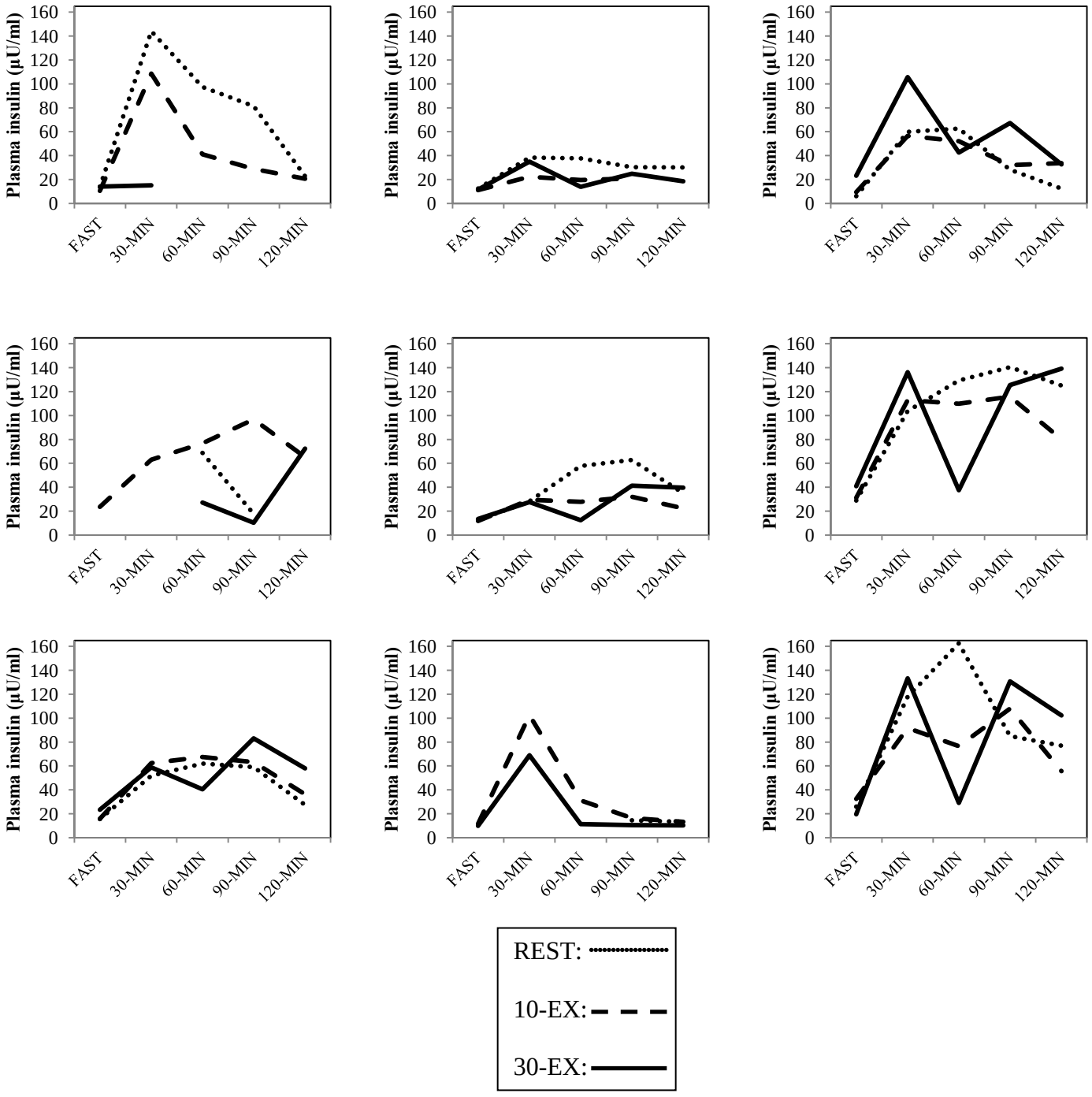
Breakfast Category	**Food Items***	**# of subjects**
Standard Breakfast (no food allergies/intolerances)	• Plain bagel • Low fat milk (1% fat) • Margarine	5
Gluten and lactose free	• Food for Life® gluten free brown rice bread • Peanut butter • Silk® original soymilk	1
Lactose free	• Plain bagel • Lactaid® low fat milk (1% fat) • Margarine	1
Lactose free (subjects preferred no milk substitute)	• Plain bagel • Nutz over Chocolate Luna Bar® • Margarine	2

* The caloric content of each meal was calculated to be 20% of each subject's estimated energy requirement determined by the Mifflin-St. Jeor equation. The amount of each food item was then adjusted so the macronutrient distribution was 55% carbohydrate, 30% fat and 15% protein for each subject. Each subjectt received an identical meal at each experimental session.

APPENDIX N

INDIVIDUAL CHANGES IN PLASMA INSULIN FOR EACH SUBJECT

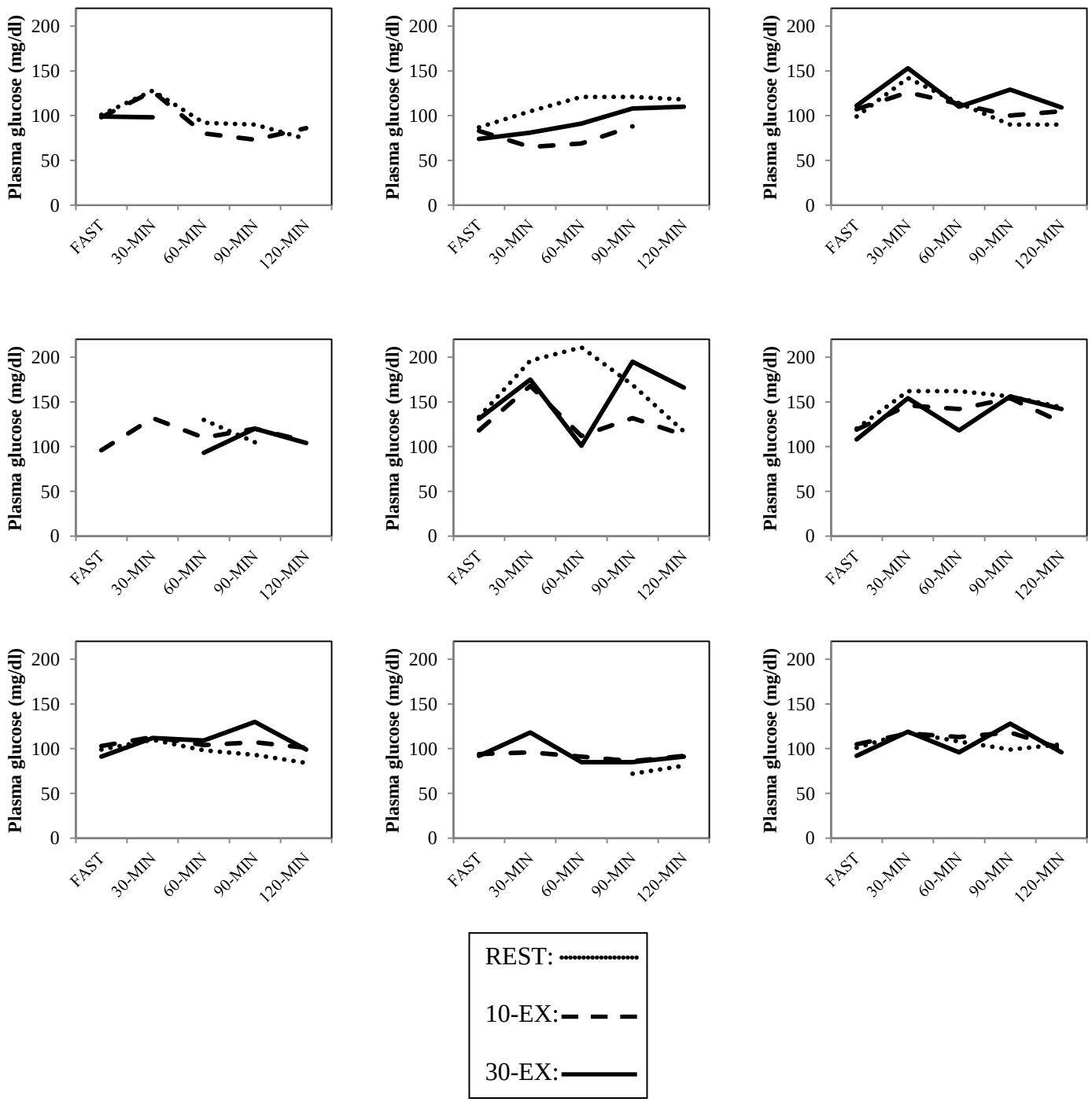
Individual changes in plasma insulin (µU/ml) for each subject



APPENDIX O

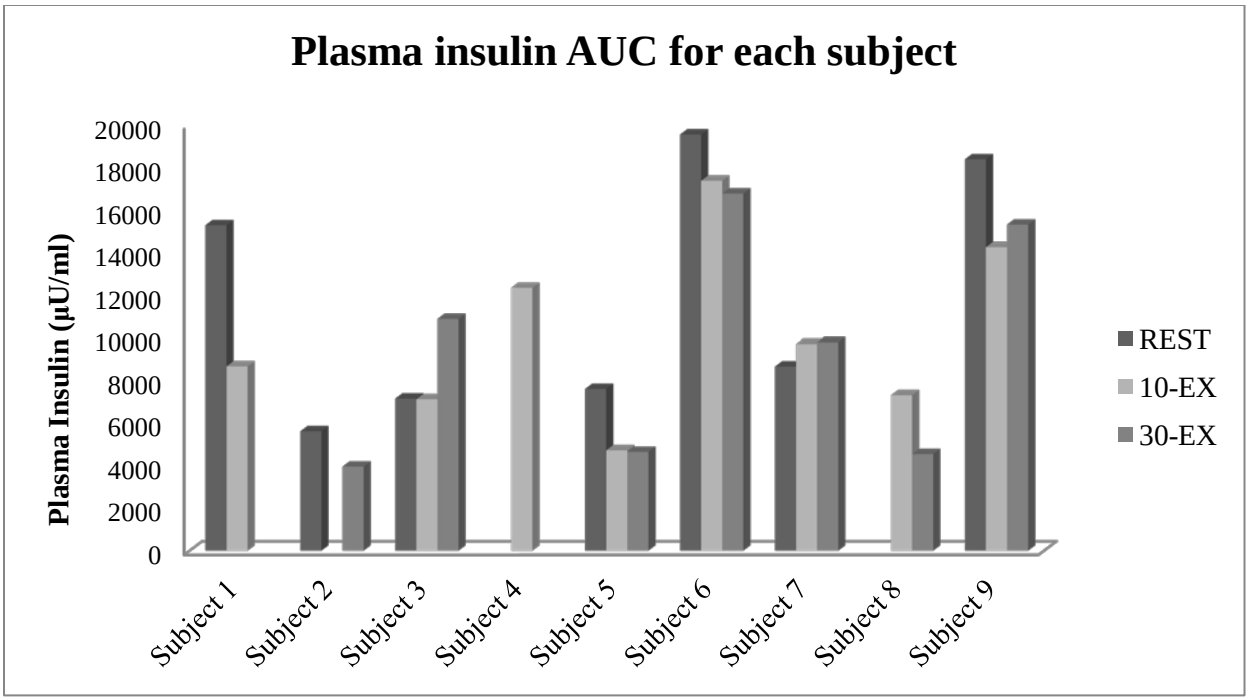
INDIVIDUAL CHANGES IN PLASMA GLUCOSE FOR EACH SUBJECT

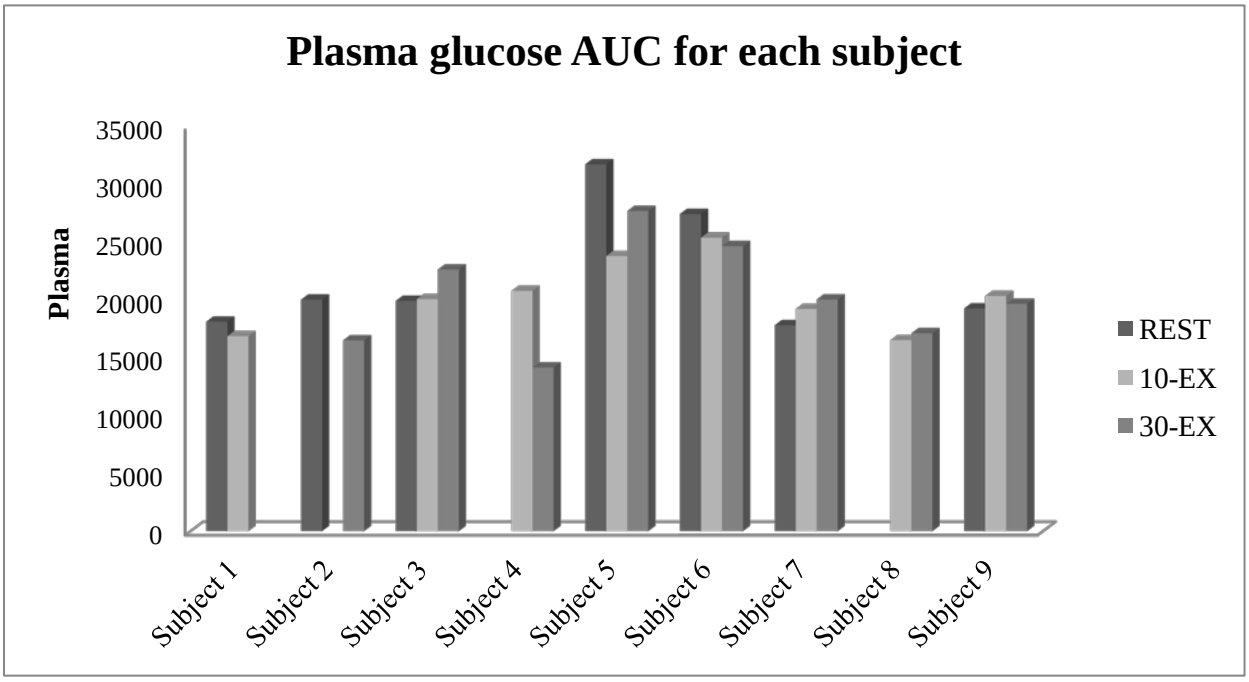
Individual changes in plasma glucose (mg/dl) for each subject



APPENDIX P

INSULIN AND GLUCOSE AUC FOR EACH INDIVIDUAL SUBJECT


Plasma insulin AUC for each subject
Plasma Insulin (µU/ml)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
REST
10-EX
30-EX


Plasma glucose AUC for each subject
Plasma
35000
30000
25000
20000
15000
10000
5000
0
Subject 1
Subject 2
Subject 3
Subject 4
Subject 5
Subject 6
Subject 7
Subject 8
Subject 9
REST
10-EX
30-EX

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